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THE THERMAL DEATH POINTS OF PATHOGENIC MICRO-ORGANISMS IN MILK

By

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THE THERMAL DEATH POINTS OF PATHOGENIC MICRO-ORGANISMS IN MILK.[a]

By Milton J. Rosenau,

Surgeon and Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

The temperature at which milk should be pasteurized hinges on the thermal death points of the pathogenic micro-organisms which contaminate it. The pathogenic micro-organisms most frequently found in market milk are those causing tuberculosis, typhoid fever, diphtheria, scarlet fever, dysentery, and Malta fever. Fortunately none of the organisms causing the above-mentioned diseases has resisting spores. Moderate degrees of heat are, therefore, sufficient to render milk safe so far as these dangers are concerned. The streptococci, staphylococci, and most of the bacteria associated with infantile diarrhea are also readily destroyed by heat.

Although it would appear to be a comparatively simple matter to determine precisely the temperature at which micro-organisms die, such work is in fact surrounded by many difficulties and pitfalls; different investigators have come to widely different results. Some of these discrepancies are only apparent and may be explained by the relation of time to temperature. The longer the time of exposure, the lower the temperature necessary to kill any organism. Differences in methods are also responsible for differences in results. Thus some workers start with the fluid containing the micro-organisms to be tested at room temperature and raise it to the desired temperature; others introduce the bacteria after the fluid has been heated.

Care must be taken in thermal death-point work to see that the entire volume of the water bath, as well as the fluid medium tested, has approximately the same temperature throughout. Unless attention to this point is given, a difference of 5° to 10° C. between the top

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[a] Manuscript submitted for publication February 11, 1908.

and bottom layers of the fluid may be found and misleading results obtained. Again, unless a reasonably accurate thermometer is used further inaccuracies may result, for ordinary thermometers not infrequently have errors of several degrees.

Among bacteria some strains or races are more resistant to heat than others. These differences, which correspond to similar known variations in all animal and vegetable species, must be taken into account.

The individual micro-organisms in any particular culture vary so far as their resistance to heat is concerned. Not all the bacteria are killed at once. Thus it will be seen from the tables that the vast bulk of the bacteria succumb at a certain temperature, while a few hardy individuals resist several degrees higher heat or several minutes longer exposure. It is evident that when we reach the point where only a few organisms remain alive in the fluid, the results may be irregular or vary, depending upon the amount of fluid transplanted into the culture medium.

The physical and chemical composition of the fluid in which the tests are made also exerts a decided influence. Some bacteria are sensitive to acid, others to alkali. If the tests are made in pure water, we have to deal with differences in osmotic pressure (plasmolysis). If the tests are made in an albuminous, viscid, or fatty medium, protecting envelopes may form and act as insulating coverings.

Evaporation takes place so rapidly from exposed fluids that the surface layer may remain cooler than the body of the liquid. This is especially a matter of concern with milk, which sometimes forms a scum above 60° C., owing to rapid evaporation of the surface layer. This scum consists of coagulated albumins in which is enmeshed much fat. The bacteria entangled in this surface pellicle may escape the heat indicated by the thermometer in the deeper layers.

A matter of very great importance is the suitability of the medium into which the bacteria are planted, as well as the temperature and the other conditions of incubation, etc. The optimum conditions may only be realized by a special study of each micro-organism tested. Controls must always show prompt and vigorous growth.

The concentration of the suspension and other differences in the conditions of the experiment account for some of the variations in the results recorded by different observers. If a test tube is used, care must be taken that the glass be thin and that the test fluid be well below the surface of the heated water. It is especially important to prevent soiling of the walls of the test tube above the fluid. This may give false results, for the reason that the pipette or instrument used to withdraw the milk or whatever fluid is used may come into contact with the upper portion of the test tube and thereby pick up

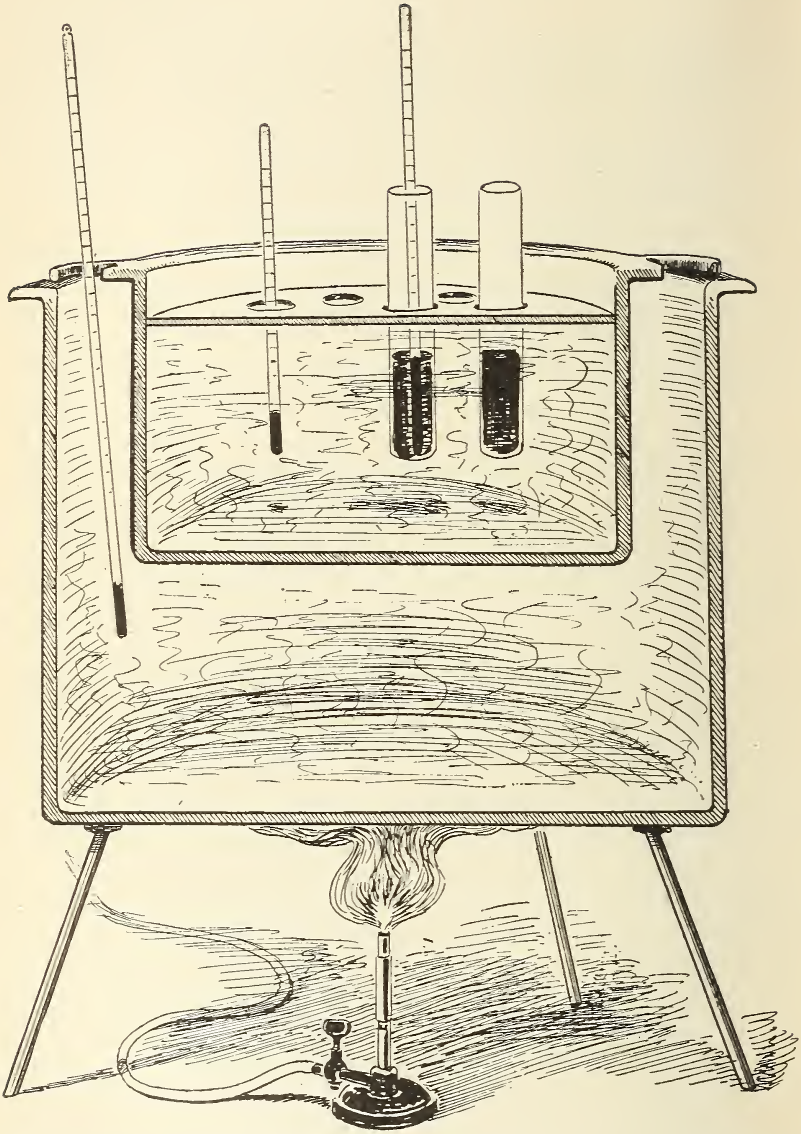

FIG. 1.—APPARATUS USED TO DETERMINE THE THERMAL DEATH POINTS OF PATHOGENIC BACTERIA IN MILK.

bacteria which may have escaped the heat, or the fluid upon the side of the test tube may afterwards flow back.

As a rule, bacteria are attenuated and lose their power to infect before they lose their ability to vegetate upon artifical culture media. It is, therefore, safe to assume that a micro-organism that will not grow in artificial media under favorable conditions is "dead." The tubercle bacillus is an exception to this rule, for reasons given further on.

METHODS.

The methods used in the tests recorded below were planned to imitate the actual conditions of pasteurization, so far as practicable, in laboratory experiments.

Except in the case of the tubercle bacillus, all the organisms were first planted in sterilized milk and not subjected to the tests until an abundant growth was obtained. In order to obtain pure cultures it was first necessary to sterilize the milk, which was done by the fractional method. It is believed that this does not appreciably influence the results obtained. By growing the micro-organisms in milk we perhaps imitate the natural conditions more closely than by using suspensions prepared for the purpose.

All the tests were made by placing the test tube containing the cool infected milk into the water bath at the desired temperature, usually 60° C. The time required for the milk to reach the temperature stated is always given.

The illustration shows how the apparatus was arranged for all the tests. The temperature was controlled within a fraction of a degree; it never varied more than half a degree, and, as a rule, was kept within one or two tenths of a degree. The test tubes containing about 10 to 15 c. c. of milk were immersed so that the level of the milk in the tube was well below the level of the water.

The reaction of the milk was determined by titration with $\frac{N}{10}$ NaOH solution, using phenolphthalein as indicator, and the results are expressed in terms of lactic acid. The reaction of the milk was always taken at the time of testing the thermal death points.[a]

Enrichment methods were used only in the case of cholera, but did not materially alter the results of the simpler methods.

The tests were made with both whole and skimmed milk. As a rule, the milk was first partly skimmed in accordance with the usual bacteriologic technic in preparing this culture fluid. Whether whole milk or skimmed milk was used is stated in each instance.

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[a] I am indebted to Dr. Joseph H. Kastle, Chief of the Division of Chemistry, for making these tests, as well as for determining the chemical composition of the milks used in the work on *B. tuberculosis*. I am also indebted to my colleagues in the Division of Pathology and Bacteriology, especially Doctors McCoy, Stimson, Miller, King and Anderson, for assistance with the tests thorughout this work. Further I desire to express my special obligations to Doctor Stimson for assistance in collecting the literature upon the thermal death point of the turbercle bacillus.

The test tubes in which the infected milk was heated were open to the air, and scum formation was disregarded in all instances, my object being to determine the thermal death point against natural difficulties, so that the results might be applied with confidence to practical pasteurization.

BACILLUS TUBERCULOSIS.

Certain special difficulties are met with in determining the thermal death point of the tubercle bacillus. This organism does not grow readily upon artificial media. The few experiments made to determine its thermal death point by cultural methods have no significance, because its vegetability upon artificial media does not correspond to its power of growing in the animal organism. It is therefore necessary to inoculate animals in order to determine whether or not the tubercle bacillus is alive and virulent. Here again we meet with complications. Dead tubercle bacilli have a certain amount of pathogenic power and produce lesions, including tubercle formation, abcesses, and coagulation necrosis. However, while we lack a criterion to determine with precision the exact point when the tubercle bacillus dies, we are able by means of animal inoculations to determine just when the tubercle bacillus is so enfeebled that it is no longer able to infect. This, after all, is the important practical point.

ORIGINAL EXPERIMENTS.

In all the tubercle experiments raw whole milk was used, the chemical composition of which is stated in each case. The number of bacteria other than the tubercle bacillus was determined by the usual method on agar plates.

The young tubercle cultures were rubbed up with the milk in a mortar until a fairly uniform suspension was obtained. Otherwise the methods used were those given on page 9.

Nine experiments were made with five different bovine cultures, the details of which will be found in the following tables. The results are consistent so far as the thermal death point is concerned. The only irregularity will be found in Table No. 1, guinea pig No. A12. This animal did not have lesions differing markedly from those caused by dead tubercle bacilli; but the secondary guinea pig (A12[1]) died as a result of generalized tuberculosis, affecting especially the lungs. The secondary guinea pig, inoculated with the lesions of A9, remained well. The other animals of this series, namely, A7, A8, A10, and A11, evidently did not have an active tuberculosis due to living and virulent tubercle bacilli.

Table No. 1.—*Series A.*

Bovine culture, H, from Leonard Pearson.

This culture was isolated July 5, 1899, from the omentum of a guinea pig that had been inoculated with an emulsion of caseous material from the mesenteric gland of a Jersey cow 7 years old. The culture was grown on blood serum for 22 generations, then transplanted on glycerin-agar. The culture used was either the thirty-third or thirty-fourth generation on glycerin-agar. The culture was virulent for guinea pigs, rabbits, and cattle.

Twenty cubic centimeters of a mixed market milk was heavily inoculated from two subcultures on potato.

Under the microscope many tubercle bacilli lying singly and in clusters were found in each field.

Composition of the milk: Specific gravity, 1.031; fat, 5.2; total solids, 13.62; solids not fat, 8.42; ash, 0.73; acidity, 0.149; refractometer, 41; sediment (by volume), 0.07; milk sugar, 4.18; no coloring matter; no preservatives.

This milk contained 840,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. of the infected milk injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
A1	Control; 28° C. (at start)	260	330	Died on 21st day	Generalized tuberculosis.	Inguinal glands on both sides enlarged and caseous; general miliary tuberculosis of peritoneum; liver enlarged and has few whitish nodules; spleen about twice normal size, no distinct tubercles visible; few tubercles in upper lobe left lung; anterior mediastinal glands swollen, not caseous.
A2	do	250	220	Died on 23d day	do	Emaciation; inguinal glands enlarged and caseated; general miliary tuberculosis of peritoneum; liver much enlarged, contains few whitish nodules; spleen four times normal size, with few whitish areas; omentum rolled up into large caseous mass; few small tubercles in lungs.
A3	60° (took 4 minutes to heat from 28° to 60°).	260	250	Died on 52d day	do	Emaciation; both inguinal and axillary glands much enlarged; nodule size of pea in mesentery; spleen four times normal size and tuberculous; liver and lungs contain advanced tubercles.
A4	1 minute at 60°	260	325	Chloroformed 55th day	do	One inguinal gland enlarged; caseous abscess in abdominal wall at site; several nodules scattered on peritoneum; omentum has numerous tubercles;

						liver and spleen both have caseous nodules; anterior mediastinal glands enlarged and caseous; lung contains one small nodule.
A5	5 minutes at 60°	265	375	do	Tuberculosis (?)	Small noncaseous nodule in abdominal wall at site; few nodules not caseous in omentum. *A secondary guinea pig* inoculated with emulsion of lesions and glands (site, omentum, spleen, liver, and lung of A5) developed generalized tuberculosis and died in 45 days.
A6	do	270	430	Died in 8 hours following injection of tuberculin (2 c. c.) on 97th day.	Generalized tuberculosis.	Omentum thick as one's little finger and caseous; celiac group of glands large and caseous; anterior mediastinal glands enlarged; yellow nodules in substance of liver, spleen, and kidney.
A7	10 minutes at 60°	275	360	Chloroformed 55th day	Negative	Inguinal glands enlarged; small nodule, not caseous, in abdominal wall at site; few noncaseous nodules in omentum.
A8	do	275	575	Chloroformed 98th day	do	Seemed slightly sick following subcutaneous inoculation 2 c. c. tuberculin, but recovered; normal throughout at autopsy.
A9	15 minutes at 60°	280	370	Chloroformed 55th day	do	Small caseous abscess at site in abdominal wall; small nodule in mesentery; several smaller nodules on peritoneum posteriorly; spleen normal but bound to surrounding parts; omentum contains numerous nodules, some caseous. *A secondary guinea pig* inoculated into the peritoneal cavity with an emulsion of the lesions and apparently normal glands of A9 (viz, site, liver, spleen, peritoneal nodules, and omentum) remained normal and gained weight; when tested with 2 c. c. tuberculin on the 108th day it did not respond; no lesions at autopsy.
A10	do	280	600	Chloroformed 98th day	do	Seemed sick, but did not die, as result of subcutaneous inoculation 2 c. c. tuberculin; few hard nodes in omentum.
A11	20 minutes at 60°	280	300	Chloroformed 55th day	do	Caseous abscess in abdominal wall at site; noncaseous nodules in omentum; spleen bound to surrounding parts, otherwise normal; nodules on diaphragmatic peritoneum; otherwise normal.
A12	do	280	300	Died in 9 hours following injection of tuberculin (2 c. c.) on 97th day.	Negative (?)	Caseous nodes in omentum; celiac group of glands size of a marble; anterior mediastinal glands enlarged; yellowish nodules in liver and spleen. *A secondary guinea pig* inoculated into the peritoneal cavity with the lesions and glands of A12 developed generalized tuberculosis, especially of the lungs; died in 54 days.

Table No. 2.—*Series B.*

Bovine culture, IXb2, from Theobald Smith.

Twenty cubic centimeters of market milk heavily inoculated from a glycerin-agar slant.

Stained smears showed numerous bacilli in each field, also a few small clumps.

Composition of the milk: Specific gravity, 1.0308; fat, 3.7; total solids, 12.3; solids not fat, 8.6; ash, 0.63; acidity, 0.15; refractometer, 40.3; sediment, 0.07; milk sugar, 4.81; no coloring matter; no preservatives.

This milk contained 2,440,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and 1,380 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
B1	Control; 25° C. (at start)	255	235	Died on 23d day	Generalized tuberculosis.	Emaciation; tuberculosis of inguinal glands, peritoneum, liver, spleen, lungs, mediastinal glands, and omentum.
B2	60° (took 4¼ minutes to heat from 25° to 60°)	255	425	Died in 7 hours following injection of tuberculin (2 c. c.) on 82d day.	do	Inguinal glands enlarged; mesenteric glands size of small marble near pylorus, not caseous; tuberculosis of spleen; anterior mediastinal gland enlarged; ileolumbar gland enlarged and caseous.
B3	1 minute at 60°	255	425	Died in 4 hours following injection of tuberculin (2 c. c.) on 82d day.	do	Emaciation; inguinal, axillary and mediastinal glands caseous; large caseous nodule at site; spleen and liver full of tubercles; nodules on peritoneum and in lungs.
B4	7 minutes at 60°	260	250	do	do	Inguinal glands large and caseous; nodules on peritoneum; spleen and liver full of tubercles; retroperitoneal glands caseous; omentum full of hard nodules; anterior mediastinal glands enlarged.
B5	10 minutes at 60°	260	315	do	do	Inguinal and mediastinal glands enlarged; nodules on peritoneum; retroperitoneal glands caseous; tubercles in liver, spleen, testicles, and lungs.
B6	12 minutes at 60°	265	425	Died in 4 hours following injection of tuberculin (2 c. c.) on 93d day.	do	Inguinal, axillary, and mediastinal glands enlarged; tubercles on peritoneum, and in spleen, liver, omentum, and lungs.
B7	15 minutes at 60°	265	500	Chloroformed 82d day	Negative	No evidence of tuberculosis at autopsy.

B8	18 minutes at 60°	265	475	Died in 12 hours following injection of tuberculin (2 c. c.) on 82d day.	do	Do.
B9	20 minutes at 60°	270	415	Died in 15 hours following injection of tuberculin (2 c. c.) on 82d day.	do	A small caseous focus in one ileolumbar gland; otherwise normal.

TABLE NO. 3.—*Series C.*

Bovine culture, IXb2, from Theobald Smith.

Twenty cubic centimeters of market milk heavily inoculated from a glycerin-agar slant.

Stained smears showed numerous bacilli in each field, also occasional clumps.

Composition of the milk: Specific gravity, 1.0308; fat, 3.7; total solids, 12.3; solids not fat, 6; ash, 0.63; acidity, 0.15; refractometer, 40.3; sediment, 0.07; milk sugar, 4.81; no coloring matter; no preservatives.

This milk contained 2,440,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and 1.380 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
C1	Control; 29° C. (at start)	300	255	Died on 19th day	Generalized tuberculosis.	Emaciation; cheesy nodule at site; tuberculosis of inguinal and axillary glands, peritoneum, omentum, lungs, and pleura.
C2	do	270	215	do	do	Emaciation; tuberculosis of inguinal, axillary, mesenteric and mediastinal glands; liver and spleen enlarged and full of nodules; omentum rolled up into one large caseating mass; tubercles in both lungs.
C3	60° (took 1½ minutes to heat from 29° to 60°).	290	250	Died on 24th day	do	Emaciation; caseous nodule at site; inguinal glands enlarged; general miliary tuberculosis of peritoneum; whitish and yellow nodule in spleen and liver; abdominal and mediastinal glands caseous; omentum rolled up into a hard mass with softened areas.
C4	65° (took 5 minutes to heat from 29° to 65°).	285	270	Died on 29th day	Negative	No evidence of tuberculosis at autopsy; inguinal glands moderately enlarged; adhesions between omentum and abdominal wall; stomach dilated; otherwise normal.
C5	1 minute at 65°	300	600	Chloroformed 93d day	do	Did not react to 2 c. c. tuberculin; pregnant; caseous nodule in omentum; otherwise normal. *Secondary guinea pig* inoculated intraperitoneally with emulsion of omental nodules, pancreas, etc., of C5 gained weight, did not respond to 2 c. c. tuberculin on 69th day; autopsy negative.

C6	5 minutes at 65°	300	550	do	do	Became sick, but did not die following tuberculin; normal at autopsy excepting a nodule, not caseous, in pancreas. *Secondary guinea pig* (same details as C5), negative.
C7	8 minutes at 65°	325	600	Died on 90th day	do	Streptococcal double pneumonia; no evidence of tuberculosis at autopsy.
C8	10 minutes at 65°	325	520	Chloroformed 93d day	do	Did not react to tuberculin; normal at autopsy except a caseous enlargement in omental pancreas, with adhesions to abdominal wall. *Secondary guinea pig* (same detail as C5), negative.
C9	15 minutes at 65°	330	520	Died in 9 hours, following injection of tuberculin (2 c. c.) on 93d day.	do	Well-preserved, pregnant; excess peritoneal fluid; small caseous abscess at site; few nodules in omentum, some softened; adhesions between liver and stomach. *Secondary guinea pig* (same details as C5), negative.

TABLE NO. 4.—*Series D*.

Bovine culture, Bovine III, from Department of Agriculture.

Twenty cubic centimeters special market milk rubbed up with culture.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, few in clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk contained 2,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

The temperature during this experiment averaged from 59.7° to 60.3° C.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
D1	Control; 24.5° C. (at start).	225	190	Died on 11th day	Generalized tuberculosis.	Emaciation; inguinal glands enlarged; 1 anterior mediastinal gland caseous; nodules on peritoneum; omentum many nodules, some size of pea, none softened; spleen fibrinous peripheric splenitis.
D2	do	230	190	Died on 27th day	do	Emaciation; excess fluid in peritoneal cavity; peritoneum studded with tubercles; caseous mass at site; omentum rolled up, large, hard, and caseous; liver, intestines, spleen, etc., bound together; lungs contained tubercles; inguinal, axillary, and mesenteric glands enlarged.
D3	60° (took 5½ minutes to heat from 24.5° to 60°).	235	335	Died in 6 hours, following injection of tuberculin (2 c. c.) on 61st day.	do	Hard nodules at site, not caseous; inguinal glands enlarged; excess clear fluid in peritoneal cavity; small nodules on peritoneal surface; omentum contains soft nodules; liver, spleen enlarged, and full of tubercles; anterior mediastinal gland enlarged; tubercle in lung.
D4	2 minutes at 60°	235	400	Died in 6 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Inguinal and axillary glands enlarged, caseous; excess clear fluid in peritoneal cavity; spleen, liver enlarged, full of tubercles; omentum, several hard caseous nodules; caseous tubercles on testicle; mediastinal and bronchial glands large, caseous; ileolumbar gland involved; tubercle in lung.
D5	5 minutes at 60°	240	365	Died in 5 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Inguinal and axillary glands enlarged; diaphragm studded with nodules; liver mottled with large yellow areas; spleen large and numerous tubercles; caseous nodules in omentum; pancreas, mediastinal, bronchial, and mesenteric glands much enlarged.
D6	10 minutes at 60°	245	450	Died in 14 hours following injection of tuberculin (2 c. c.) on 76th day.	Negative	Excess clear fluid in peritoneal cavity; few hard nodules, one caseous, in omentum; bronchial glands enlarged; otherwise normal.
D7	15 minutes at 60°	250	380	Died in 15 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Moderate enlargement of inguinal glands; peritoneal fluid excessive; caseous nodules in omentum and caseous abscess between spleen and kidney; small caseous abscess at site of inoculation; bronchial glands slightly enlarged.

						Secondary guinea pig inoculated intraperitoneally with emulsion of lesions of D7 remained well, did not respond to 2 c. c. tuberculin on the 71st day, gained weight; no lesions at autopsy.
D8	18 minutes at 60°	250	.....	Died on 5th day	do	Death due to peritonitis resulting from intestinal perforation.
D9	20 minutes at 60°	250	500	Died in 7 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Slight excess clear fluid in peritoneal cavity; omentum contains a caseous nodule; perisplenitis; otherwise normal. *Secondary guinea pig* (see D7), negative.
D10	23 minutes at 60°	250	550	Chloroformed 76th day	do	Seemed sick as a result of tuberculin injection, but recovered; no lesions at autopsy.
D11	25 minutes at 60°	250	205	Died on 15th day	do	Omentum contains few small nodules, not caseous; slight enlargement of inguinal, axillary, and mediastinal glands; all else normal.
D12	28 minutes at 60°	250	475	Died in 6 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Not emaciated; caseous nodule at site; excess clear fluid in peritoneum; few small nodules in omentum; otherwise normal.
D13	30 minutes at 60°	250	440	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Caseous nodule in pancreas and omentum; nodule on peritoneal surface of diaphragm. *Secondary guinea pig* (same details as D7), negative.
D14	35 minutes at 60°	255	500	Chloroformed 76th day	do	Became sick following tuberculin reaction, but recovered; nothing abnormal at autopsy excepting a few grayish patches on spleen not resembling tubercles. *Secondary guinea pig* (same details as D7), negative.
D15	40 minutes at 60°	260	460	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Omental pancreas thickened and contains caseous lumps, some 3 mm. in diameter; patches of perisplenitis and one perinephritis. *Secondary guinea pig* (same details as D7), negative.
D16	45 minutes at 60°	265	430	Died in 16 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Caseous nodules in pancreas and omentum; caseous abscess over right kidney; small hard nodule on diaphragm. *Secondary guinea pig* (same details as D7), negative.
D17	50 minutes at 60°	265	520	Died in 12 hours following injection of tuberculin (2 c. c.) on 76th day.	do	Omental pancreas enlarged and nodular; one ileolumbar gland and inguinal glands enlarged, but not caseous; whitish area on edge of spleen; otherwise normal. *Secondary guinea pig* (same details as D7), negative.
D18	60 minutes at 60°	265	500	Chloroformed 76th day	do	Failed to react to subcutaneous injection of 2 c. c. tuberculin; nothing abnormal found at autopsy.

Table No. 5.—*Series E.*

Bovine culture, Bovine III, from Department of Agriculture.

Twenty cubic centimeters special market milk rubbed up with culture.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, few in clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk contained 2,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

The temperature during this experiment averaged from 64.8° to 65.1° C.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
E1	Control; 24.5° C. (at start).	265	235	Died on 15th day	Generalized tuberculosis.	Emaciation; large caseous mass each testicle; caseous nodules in seminal vesicles; generalized miliary tuberculosis of peritoneum; omentum rolled up and contains many hard yellow nodules, some size of pea; spleen enlarged; anterior mediastinal gland caseous; inguinal glands enlarged.
E2	do	270	250	do	Generalized tuberculosis (?).	No autopsy.
E3	60° (took 2¼ minutes to heat from 24.5° to 60°).	270	240	Died on 23d day	Generalized tuberculosis.	Inguinal and axillary glands enlarged; great numbers of tubercles scattered over peritoneal surface; masses and nodules in omentum and near site; spleen and both lungs contain tubercles.
E4	65° (took 5 minutes to heat from 24.5° to 65°).	270	450	Chloroformed 77th day	Negative	Did not react to subcutaneous injection of 2 c. c. tuberculin; well nourished; caseous abscess in abdominal wall and at site; many large caseous areas in omentum; mesenteric glands enlarged, anterior mediastinal slightly; pregnant. *A secondary guinea pig* inoculated intraperitoneally with an emulsion of lesions and glands of E4 remained well, gained weight, failed to respond to 2 c. c. tuberculin on the 68th day; autopsy negative.

E5	2 minutes at 65°	280	350	Died in 5 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in diaphragm and omentum; excess of peritoneal fluid. *Secondary guinea pig*, negative.
E6	3 minutes at 65°	280	400	Died in 17 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in omentum; same in region of each ovary; hard, noncaseous nodules on under surface of diaphragm; bronchial glands enlarged. *Secondary guinea pig*, negative.
E7	5 minutes at 65°	285	195	Died in 12 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Omentum reduced to cheesy sac; large nodule on diaphragm; bronchial glands enlarged; few small yellow patches in liver and one in spleen. *Secondary guinea pig*, negative.
E8	8 minutes at 65°	285	480	Died in 13 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Nothing abnormal found at autopsy. Secondary guinea pig (same as E4), negative.
E9	10 minutes at 65°	290	445	do	do	Caseous nodule in omentum; otherwise normal. *Secondary guinea pig*, negative.
E10	12 minutes at 65°	290	425	Chloroformed 77th day	do	Did not react to subcutaneous injection of 2 c. c. tuberculin; nodules on diaphragm, pleura; mediastinal glands enlarged, not caseous; omentum thickened, but no nodules; no caseation except glandular enlargements; no definite findings. *Secondary guinea pig*, negative.
E11	15 minutes at 65°	300	490	Died in 10 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous abscess at site; caseous nodules in omentum and between liver and intestine. *Secondary guinea pig* died in 7 days from septic peritonitis; no evidence of tuberculosis.
E12	20 minutes at 65°	260	400	Died in 13 hours following injection of tuberculin (2 c. c.) on 77th day.	do	Caseous nodules in omentum, 1 in spleen, and between spleen and kidney, and between kidney and liver; all else normal. *Secondary guinea pig*, negative.

TABLE No. 6.—*Series F.*

Bovine culture, B I, from Trudeau, Saranac Laboratory.

This culture was obtained from a cheesy mass in the apex of left lung of a cow reacting to tuberculin test at 41.1° C. on September 28, 1902; age of cow, 18 to 20 years.

Some of the caseous material inoculated into guinea pigs and the culture recovered from the inguinal glands of the guinea pigs on Löffler's sheep serum, subculture on cow serum.

A moderately heavy suspension was rubbed up in a sample of mixed market milk.

Upon smears some bacilli were found in each field, lying singly and in clusters.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
F1	Control; 28° C. (at start)	265	315	Died in 5 hours following injection of tuberculin (2 c. c.) on 59th day.	Generalized tuberculosis.	Emaciation and very extensive generalized tuberculosis of all organs, except lungs; inguinal glands caseous.
F2	.....do	250	450	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	.....do	Fairly well preserved; pronounced generalized tuberculosis; 1 inguinal gland caseous; abscess at site; spleen and omentum contain many tubercles, also liver; mediastinal and hepatic glands caseous; areas in lung perhaps tuberculous.
F3	60° (took 4 minutes to heat from 28° to 60°).	280	475	Died in 5 hours following injection of tuberculin (2 c. c.) on 59th day.	.....do	Inguinal glands enlarged and caseous; axillary glands enlarged; caseous nodule at site; spleen very large and filled with tubercles; omentum has numerous hard and caseous nodules; tubercles in liver; anterior mediastinal gland enlarged, also bronchial glands which are caseous.

F4	1 minute at 60°	280	500	Died in 20 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well-preserved caseous abscess in abdominal wall at site; few small, hard caseous nodules in omentum; tubercles in spleen, which is bound down; liver contains disseminated tubercles; bronchial glands enlarged and caseous.
F5	2 minutes at 60°	275	440	Died in 19 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Omentum contains hard nodules, not caseous; tubercles on spleen; few nodules on surface of liver; bronchial glands enlarged.
F6	3 minutes at 60°	290	475	Chloroformed 60th day	Negative	Did not react to tuberculin injection (2 c. c.) subcutaneously; normal at autopsy except small yellow nodule on spleen.
F7	4 minutes at 60°	270	430	Died in 14 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well nourished; hard subsplenic nodule; omentum nodular; no caseation; nothing else abnormal.
F8	5 minutes at 60°	250	465	do	do	Few hard nodules in omentum, none caseous; otherwise normal.
F9	7 minutes at 60°	280	445	Chloroformed 60th day	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; few hard caseous nodules in omentum; otherwise normal.
F10	10 minutes at 60°	320	510	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; 1 small hard nodule in omentum; no caseation; otherwise normal.
F11	15 minutes at 60°	335	400	do	do	Did not react to tuberculin injection (2c. c.) subcutaneously; 1 large caseous nodule in omentum.
F12	20 minutes at 60°	280	480	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; hard nodules in omentum; no caseation; perisplenitis; otherwise normal.
F13	30 minutes at 60°	310	550	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well nourished; small caseous abscess in abdominal wall and at site; small patch perisplenitis; few nodules in omentum, one caseous; bronchial glands enlarged.
F14	40 minutes at 60°	270	525	Chloroformed 60th day	do	Did not react to tuberculin injection (2c. c.) subcutaneously; well preserved, nothing abnormal found at autopsy.
F15	50 minutes at 60°	265	440	Died in 18 hours following injection of tuberculin (2 c. c.) on 59th day.	do	Well preserved; caseous nodule about the size of a pea in right seminal vesicle; several hard and caseous tubercles in omentum; spleen adherent; 1 mesenteric gland caseous; otherwise normal.

TABLE No. 7.—*Series G.*

Bovine culture, H Rav, from the Rockefeller Institute for Medical Research.

This culture was kindly sent me by Dr. Flexner, who states that it was isolated from the mesenteric gland of a cow.

A heavy suspension rubbed up in a small quantity of mixed market milk.

Stained smears showed numerous bacilli in each field and also numerous clumps of various sizes.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41.0; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
G1	Control; 27° C. (at start)...	310	265	Died on 18th day.........	Generalized tuberculosis.	Emaciation; generalized miliary tuberculosis; deposit upon peritoneum and tunica vaginalis; omentum rolled up and contains many large and small nodules; spleen enlarged and tuberculous; anterior mediastinal glands caseous; caseous abscess at site in abdominal wall; femoral glands enlarged.
G2	.....do.....................	315	340	Died on 29th day.........	.....do................	Emaciation; caseous abscess in abdominal wall at site; superficial glands enlarged; peritoneum studded with nodules, contains sanious fluid; omentum one large hard and caseous mass; tubercles in spleen and lungs; mediastinal glands enlarged.
G3	60° (took 4½ minutes to heat from 27° to 60°).	270	550	Died in 6 hours following injection of tuberculin (2 c. c.) on 63d day.	Localized tuberculosis.	Well preserved; omentum contains few hard and caseous nodules; ileolumbar gland size of pea and caseous; mesenteric glands enlarged, not softened; glands and celiac axis enlarged and size of filbert, caseous.
G4	1 minute at 60°.............	290	400	Died on 44th day.........	Negative............	Streptococcal double pneumonia; well preserved; one caseous gland in axilla; few hard nodules in omentum; one retroperitoneal gland caseous; otherwise normal.

G5	2 minutes at 60°	320	495	Died in 6 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Omentum contains few small nodules, one caseous; ileolumbar glands enlarged and caseous; glands in region of celiac axis enlarged and caseous; few mesenteric glands enlarged, but not caseous.
G6	3 minutes at 60°	280	375	Died on 31st day	do	Double streptococcal pneumonia; well preserved; no lesions indicating tuberculosis at autopsy.
G7	4 minutes at 60°	265	575	Died in 19 hours following injection of tuberculin (2 c. c.) on 65th day.	do	No indications of tuberculosis at autopsy.
G8	5 minutes at 60°	260	225	Died on 16th day	do	Do.
G9	7 minutes at 60°	325	285	Died on 14th day	do	Double pneumonia (streptococcal); few whitish firm noncaseous nodules posteriorly under peritoneum.
G10	10 minutes at 60°	290	450	Died in 18 hours following injection of tuberculin (2 c. c.) on 64th day.	do	Left inguinal glands enlarged to size of marble and caseous; few hard nodules in omentum.
G11	15 minutes at 60°	320	480	Died in 11 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Few nodules in omentum; whitish area in spleen; otherwise normal. *Secondary guinea pig* inoculated intraperitoneally with emulsion of lesions of G11 remained well, gained weight, did not respond to 2 c. c. tuberculin on the 65th day; no lesions at autopsy.
G12	20 minutes at 60°	275	350	Died on 50th day	do	Abscess of heart, which contained a large mass occupying whole lower half, yellowish, fluctuating; cocci and bacilli in lymph nodes and spleen smears negative for tubercle bacilli; no evidence of tuberculosis.
G13	30 minutes at 60°	280	490	Died in 13 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Omentum nodular; anterior mediastinal glands enlarged on right side, not caseous; perisplenitis. *Secondary guinea pig* inoculated intraperitoneally with emulsion of lesions; of G13 remained well, gained weight, did not respond to 2 c. c. tuberculin; no lesions at autopsy.
G14	40 minutes at 60°	265	450	Chloroformed 65th day	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; small caseous nodule in abdominal wall at site of inoculation; inguinal glands slightly enlarged; perisplenitis; otherwise normal.
G15	50 minutes at 60°	330	550	do	do	Did not react to tuberculin injection (2 c. c.) subcutaneously; pregnant; abortion; large deep yellow patch on liver; all else normal.

TABLE NO. 8.—*Series H.*

Bovine culture, H Rav, from the Rockefeller Institute for Medical Research.

This culture was kindly sent me by Dr. Flexner, who states that it was isolated from the mesenteric gland of a cow.

A heavy suspension rubbed up in a small quantity of mixed market milk.

Stained smears showed numerous bacilli in each field and also numerous clumps of various sizes.

Composition of the milk: Specific gravity, 1.03; fat, 4.8; total solids, 13.5; solids not fat, 8.7; ash, —; acidity, 0.149; refractometer, 41; sediment, 0.07; milk sugar, —; no coloring matter; no preservatives.

This milk contained 880,000 bacteria (other than tubercle bacilli) per cubic centimeter just before it was used for this experiment and none at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
H1	Control; 31° C. (at start)	250	265	Died on 38th day	Generalized tuberculosis.	Emaciation; caseous nodule at site; inguinal and axillary glands enlarged; peritoneum studded with tubercles; spleen enlarged with tubercles; omentum one large hard caseous mass; liver full of tubercles; lungs contains few nodules; mediastinal glands enlarged.
H2	do	290	210	Died on 35th day	do	Much emaciation; caseous mass at site; inguinal glands enlarged; peritoneum studded with miliary tubercles; liver and spleen enlarged with advanced tuberculosis; omentum large hard caseous mass; tubercles in lungs.
H3	60° (took 5 minutes to heat from 31° to 60°).	255	410	Died in 8 hours following injection of tuberculin (2 c. c.) on 63d day.	Negative	Hard noncaseous nodes in omentum; otherwise normal.
H4	1 minute at 60°	315	440	Chloroformed 64th day	do	Did not die as result of subcutaneous injection 2 c. c. tuberculin; noncaseous node in omentum; otherwise normal.
H5	3 minutes at 60°	290	410	do	do	Did not react to subcutaneous injection 2 c. c. tuberculin; omentum somewhat shrunken; otherwise normal throughout.

H6	do	320	520	Died in 8 hours following injection of tuberculin (2 c. c.) on 63d day.	do	Nothing abnormal found at autopsy.
H7	4 minutes at 60°	290	470	Died in 14 hours following injection of tuberculin (2c.c.) on 63d day.	do	Cheesy nodules in omentum; all else normal.
H8	5 minutes at 60°	260	320	Died in 10 hours following injection of tuberculin (2 c.c.) on 63d day.	do	Omentum somewhat nodular; no other evidence of tuberculosis. *A secondary guinea pig* inoculated with an emulsion of the lesions from H_8 remained normal, gained weight; no evidence of tuberculosis at autopsy; chloro formed 30th day on account of submixillary abscess (streptococci).
H9	7 minutes at 60°	255	350	do	do	Few nodules in omentum; anterior mediastinal glands enlarged; no other lesions.
H10	10 minutes at 60°	305	440	Chloroformed 64th day	do	Did not react to subcutaneous injection 2 c. c. tuberculin; few hard nodes in omentum; otherwise normal.
H11	15 minutes at 60°	270	485	do	do	Did not react to subcutaneous injection 2 c. c. tuberculin; no lesions.
H12	20 minutes at 60°	265	450	do	do	Did not react to subcutaneous injection 2 c. c. tuberculin; few nodes in omentum; spleen bound down by extensive adhesions; liver dotted with fine yellow spots; slight enlargement of ileolumbar glands; otherwise normal; pregnant. *Secondary guinea pig* inoculated peritoneally with lesion and organs of H_{12} gained weight; did not respond to tuberculin on 64th day; no lesions at autopsy.
H13	30 minutes at 60°	290	430	Died in 13 hours following injection of tuberculin (2c.c.) on 63d day.	do	Few small nodes in omentum; perisplenitis; anterior mediastinal glands enlarged.
H14	40 minutes at 60°	310	455	Chloroformed 64th day	do	Did not react to subcutaneous injection 2 c. c. tuberculin; few small omental nodes; otherwise normal throughout; pregnant.
H15	50 minutes at 60°	260	420	Died in 10 hours following injection of tuberculin (2c.c.) on 63d day.	do	Omentum slightly thickened, but not nodular; spleen adherent posteriorly; no evidence of tuberculosis.

Table No. 9.—*Series J.*

Bovine Culture, Bovine III, from Department of Agriculture.

Ten-day old culture of glycerin potassium biphosphate broth. The entire pellicle from a 100 c. c. Erlenmeyer flask rubbed up with 15 c. c. of a special (certified) milk.

Stained smears of the milk thus infected showed numerous bacilli lying separately in every field, also stray clumps and clusters.

Composition of the milk: Specific gravity, 1.0321; fat, 4.2; total solids, 12.9; solids not fat, 8.7; ash, 0.63; acidity, 0.149; refractometer, 41; sediment, —; milk sugar, 4.83; no coloring matter; no preservatives.

This milk had a reaction of 0.153 per cent acidity and contained 9,000 bacteria (other than tubercle bacilli) just before it was used for this experiment and 20 at the conclusion of the test.

About 0.5 c. c. injected into the peritoneal cavity of each guinea pig.

No. of guinea pig.	Temperature and time of heating milk.	Weight in grams of guinea pig.		Time and mode of death.	Results.	Remarks.
		Initial.	Final.			
J1	Control; 25° C. (at start)	280	200	Died on 20th day	Generalized tuberculosis.	Emaciation; inguinal glands enlarged; small caseous abscess at site; miliary tubercles studded over peritoneum; tubercles in liver, spleen, lungs; omentum rolled up into hard fibrous and caseous mass.
J2	.....do	275	225	.....do	.....do	Emaciation; inguinal and axillary glands enlarged; small node at site; miliary tubercles on peritoneum; tubercles in liver, spleen, mediastinal glands, and lungs; omentum rolled up into hard fibrous and caseous mass.
J3	.....do	270	200	Died on 19th day	.....do	Emaciation; axillary glands enlarged; large caseous abscess at site; miliary tubercles on peritoneum; tubercles in liver, spleen, and lungs.
J4	59.7° (took 5 minutes to heat from 25° to 59.7°).	285	290	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	.....do	Emaciation; inguinal glands enlarged; caseous abscess in abdominal wall at site; omentum contains large caseous nodules; tubercles in spleen, which is enlarged and bound down; liver, peritoneum, ileolumbar glands, celiac group, mesentery, anterior mediastinal glands, and lungs contain nodes.
J5	60° (took 6 minutes to heat from 25° to 60°).	260	305	Died in 8 hours following injection of tuberculin (2 c. c.) on 36th day.	.....do	Caseous nodules at site; large caseous areas in spleen, liver, and omentum; anterior mediastinal, celiac group, and retroperitoneal glands enlarged.

J6	1 minute at 60°	270	335	Died in 4 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Right inguinal gland enlarged and caseous; few small nodules on peritoneum; tubercles in spleen, liver, omentum (caseous), ileolumbar glands, celiac group (size of marble and caseous), mesenteric nodes, anterior mediastinal glands, and lungs.
J7	2 minutes at 60°	270	360	Died in 8 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Inguinal gland somewhat enlarged, red; nodules upon surface of diaphragm, in omentum and spleen; surface plaques on spleen and liver; mesenteric and anterior mediastinal glands enlarged.
J8	3 minutes at 60°	265	360	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Small caseous nodule at site; nodules in omentum, some caseous; tubercles in spleen (enlarged), one on edge of liver; lumbar glands, celiac group, mesenteric and mediastinal glands enlarged.
J9	4 minutes at 60°	265	305	Chloroformed 37th day	Negative	Perisplenitis; omentum rolled up and contains many caseous nodules; no definite glandular enlargement; liver has numerous fine light areas, not like tubercles; otherwise normal.
J10	5 minutes at 60°	285	390	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Omentum contains nodes, some caseous; spleen bound down, otherwise normal; liver contains many tubercles (?); celiac group enlarged, not caseous; lumbar glands caseous; mesenteric nodes enlarged.
J11	6 minutes at 60°	250	350	Died in 10 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Small caseous nodule at site; noncaseous nodule in omentum; few yellow areas in substance of spleen and liver; ileolumbar and celiac group enlarged; otherwise normal.
J12	8 minutes at 60°	280	335	Died in 13 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Nodule on under surface of diaphragm and in omentum, not caseous; patches perisplenitis and hepatitis; retroperitoneal glands enlarged; otherwise normal.
J13	10 minutes at 60°	290	420	Died in 10 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Nodules on under surface of diaphragm; caseous tubercles in omentum; surface patches on spleen; retroperitoneal, celiac group, and anterior mediastinal glands enlarged.
J14	12 minutes at 60°	250	360	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Few small nodules, not caseous, in omentum; lumbar glands slightly enlarged; spleen bound down posteriorly; one anterior mediastinal gland slightly enlarged; otherwise normal.
J15	15 minutes at 60°	260	270	Chloroformed 37th day	do	Did not react to subcutaneous injection 2 c. c. tuberculin; nothing abnormal at autopsy.
J16	20 minutes at 60°	250	305	Died in 6 hours following injection of tuberculin (2 c. c.) on 36th day.	do	Caseous abscess at site in abdominal wall; few small nodules, 1 caseous, in omentum; perisplenitis; liver bound to anterior abdominal wall; otherwise normal.

Conclusion.—From these experiments it is evident that the tubercle bacillus in milk loses its infective properties for guinea pigs when heated to 60° C. and maintained at that temperature for twenty minutes, or to 65° C. for a much shorter time.

It should be remembered that the milk in these tests was very heavily infected with virulent cultures, indicated by the prompt deaths of the control animals. Milk would practically never contain such an enormous amount of infection under natural conditions. It is justifiable to assume that if 60° C. for twenty minutes is sufficient to destroy the infectiveness of such milk when injected into the peritoneal cavity of a guinea pig, any ordinary market milk after such treatment would be quite safe for human use by the mouth so far as tubercle bacilli are concerned.

THE EFFECTS OF DEAD TUBERCLE BACILLI.

In the foregoing work endeavor was made to distinguish the lesions produced by the dead tubercle bacilli. For comparison a number of animals were inoculated with milk containing dead tubercle bacilli (killed at 100° C.). The necropsy notes of this series, given in Table No. 10, are very instructive, especially when compared with the foregoing work (Tables 1–9).

Prudden and Hodenphyl [a] found "that tubercle bacilli which have been killed by boiling or otherwise, when introduced into the body of the rabbit either beneath the skin or into the serous cavities, or into the blood vessels and the air spaces of the lungs, are capable, as they slowly disintegrate, of stimulating the cells of the tissues where they lodge to proliferation, and to the production of new tissue morphologically similar to tubercle tissue in its various phases. Necrosis of the new-formed cells may occur, but this differs in some respects from the coagulation necrosis induced under the usual conditions. Dead tubercle bacilli are also markedly chemotactic and capable of causing local suppuration and abscess."[b]

James Miller found that[c] "Dead tubercle bacilli can, then, produce changes which in their early stages are the same as those produced by the living organism. They differ in acuteness only. When these dead bacilli are introduced into the body they attract the leucocytes of both varieties. The cells take up the organism, the polymorphonuclear forms degenerate; the mononuclear cells are, however, capable of further development, and, probably with the assistance of connective tissue cells, form the epithelioid and giant cells. Inclosed within their cell prison the bacilli undergo a gradual disintegration, setting free a poison which may produce partial death of a cell, or, if in sufficient quantity, may produce the death of many cells. There is not the same activity in the leucocytes, nor the same vacuolation of the protoplasm and development of pseudopodia in the mononuclear forms. The epithelioid cells have not the same size or the same branched appearance, the giant cells are more rounded and smooth. The whole process gives one the impression of less activity. In the later stages the difference is more marked. There is not the same wide distribution and extension of the process, nor is there the same constant addition of new phagocytes at the margin which will become epithelioid cells and help to swell the giant cell. There is, however, in these later stages the same impression of a partial cell necrosis. The bacilli disappear, but the cells persist and show the peculiar hyaline appearance. Further, there is the same polymorphonuclear leucocytosis in the last stage of pseudo-tubercle that we have seen in true tubercle."

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[a] For further details concerning the effects of dead tubercle bacilli in the body see Prudden & Hodenphyl, N. Y. Med. Journ., June 6 and 20, 1891, and Prudden, ibid, Dec. 5, 1891. For summary of later work, with bibliography, see Herxheimer, Ziegler's Beitr., vol. 33, p. 363; also, Miller, Journ. Path. and Bact., vol. 10, 1905, p. 351.

[b] Delafield, Francis, and Prudden, T. Mitchell: A Text-book of Pathology, 8th ed. New York, Wm. Wood & Co., 1907. 1057 p. Illus. 8°.

[c] Miller, James: "The Histogenesis of the Tubercle" Journal of Pathology and Bacteriology, 1905, vol. 10, pp. 1–48.

In my own experiments, in order to avoid the confusion resulting from the effects produced by dead tubercle bacilli, doubtful lesions were carried over into another animal. These "secondary" guinea pigs appear in Tables Nos. 1 to 9. The method used was as follows: The lesions, and sometimes the organs, were ground up in a mortar with salt solution, strained through gauze, and the extract thus obtained injected into the peritoneal cavity of a young normal guinea pig. Only two of these secondary guinea pigs developed generalized tuberculosis. The others remained well, gained weight, failed to respond to tuberculin, and gave no evidence of an active tuberculosis at necropsy. Some of these secondary animals had small tuberclelike masses in the omentum, due to absorption of the products introduced into the peritoneal cavity.

Almost all the animals were tested with tuberculin in the hope that this test might distinguish between the lesions of live and of dead tubercle bacilli. This, however, was found not to be the case, for when the eight animals with lesions caused by tubercle bacilli known to be dead, were injected with 2 c. c. of tuberculin (O. T.) (see Table No. 10), three of them died. The series of guinea pigs in this table is instructive further in that it illustrates that dead tubercle bacilli in milk, when injected into the peritoneal cavity, may produce very extensive tubercular lesions with coagulation necroses (caseation), etc.

TABLE No. 10.—*Results of injecting dead tubercle bacilli in milk into the peritoneal cavity of guinea pigs.*

No. of guinea pig.	Date of inoculation.	Tubercle bacilli in milk heated to—	Culture.	Weight in grams.		Subcutaneous inoculation 2 c. c. tuberculin.		Date and manner of death.	Necropsy notes.
				Initial.	Final.	Date.	Result.		
D19	Sept. 12, 1907	100° C. 1 hour...	Bovine III...	270	325	Nov. 7, 1907	Sick; recovered.......	Chloroformed Nov. 8, 1907..	Moderate hemorrhagic local reaction at site of tuberculin inoculation; no edema; hard nodules in omentum, not caseous; perisplenitis; ileolumbar and inguinal glands slightly enlarged.
D20	.....do.......	100° 1 hour......	.....do........	265	490	Nov. 12, 1907	.....do................	Chloroformed Nov. 13, 1907.	Very marked hemorrhagic and edematous reaction at site of tuberculin inoculation; no glandular enlargements; few hard nodules in omentum; pregnant.
F16	Sept. 9, 1907	100° 30 minutes.	B1 Saranac...	260	500	Nov. 7, 1907	No effect.............	Chloroformed Nov. 8, 1907..	Enlarged mesenteric glands, not caseous.
G16	.....do.......	100° 10 minutes.	H Rav, Rockefeller Institute.	280	465	Oct. 25, 1907	Sick; recovered.......	Chloroformed Oct. 26, 1907..	Edema and hemorrhagic reaction at site of tuberculin inoculation; fine miliary nodules like tubercles on peritoneum; liver mottled with fine tubercle-like areas; omentum contains nodules, not caseous.
J17	Oct. 7, 1907	100° 1 hour.....	Bovine III...	255	355	Nov. 12, 1907	Died in 8 hours.......	....	Emaciation; hemorrhagic and edematous reaction at site of tuberculin inoculation; inguinal glands slightly enlarged and reddened; caseous nodule in abdominal wall at site of inoculation; peritoneum smooth; excess fibro-serous fluid in peritoneal cavity; omentum rolled up into large tuberculous mass containing numerous caseous foci; perisplenitis; cœliac

								group of glands enlarged, not caseous; mesenteric nodes enlarged; numerous whitish nodules on under surface of diaphragm; anterior mediastinal glands enlarged and caseous.
J18	do	do	do	255	375	do	Died in 10 hours		Moderate hemorrhagic and edematous reaction at site of tuberculin inoculation; excessive fluid in peritoneal cavity; omentum contains few small caseous nodules; spleen bound down; otherwise normal.
J19	do	do	do	280	375	do	No effect	Chloroformed Nov. 13, 1907.	Marked hemorrhagic local reaction; no glandular enlargements; nodules on under surface of diaphragm and in omentum; liver and spleen contain tubercle-like areas.
J20	do	do	do	250	350	do	Died in 12 hours		Hemorrhagic and edematous reaction at site of tuberculin inoculation; inguinal glands, both sides, enlarged and reddened; axillary glands less so; excessive clear fluid in peritoneal cavity; tendency to coagulate; small caseous nodule in abdominal wall at site; omentum rolled up and contains numerous nodules, some caseous; spleen bound down; caseous mass size of pea between spleen and kidney; spleen enlarged and contains four nodules on surface; ileolumbar glands slightly enlarged; under surface of diaphragm roughened with numerous whitish nodules; anterior mediastinal glands enlarged and caseous.

REVIEW OF THE WORK OF OTHERS UPON THE THERMAL DEATH POINT OF THE TUBERCLE BACILLUS.

The early work upon the thermal death point of the tubercle bacillus is clouded in the obscurity of meager data and insufficiently controlled and imperfectly oriented experiments.

Martin,[a] 1882, concludes, from two experiments with fragments of lung of a tuberculous guinea pig, that with an exposure to a moist temperature of 80° C. the infectivity of tubercle bacilli is not lost.

May,[b] 1883, undertook to determine how long the milk of tuberculous cows must be boiled to render it surely innocuous. He experimented with injections of material into the peritoneal cavity of guinea pigs. All the injections of cooked fluids, milk, etc., gave negative results. Most of the experiments with raw milk of tuberculous cows also gave negative results; a few positive. He concludes that there is small danger of infection from raw milk, and none at all from cooked milk, for human beings.

Sormani,[c] 1884, is reported by De Man to have injected milk containing tubercle bacilli into guinea pigs after having heated it ten minutes at 70°, 80°, and 90° C., with the result that all the animals became tuberculous. When, however, the milk was kept seething for five minutes, cooled, and injected, the animals remained sound.

Schill and Fischer,[d] 1884, studied the action of a temperature of 100° C. upon tuberculous sputum. They found that 100° of dry temperature did not surely destroy the virulence of tuberculous sputum whether dry or moist.

Voelsch[e] concluded that boiling did not destroy the virulence in tuberculous products from rabbits, serum cultures, or tuberculous sputum. His experiments were made upon rabbits, and it appears that he misinterpreted the lesions produced by dead tubercle bacilli. He went so far as to believe that boiling twice, while it attenuates, does not destroy the virulence of the tubercle bacillus.

Yersin,[f] 1888, tested the thermal death point of tubercle bacilli "with spores and without spores." The tubercle bacilli "without spores" were obtained from spleen pulp of a rabbit dead of the intravenous injection of a culture. Some of this spleen pulp, very rich in bacilli, was drawn into capillary tubes hermetically sealed and plunged into a water bath kept at the desired temperature. After heating, the pulp was inoculated upon glycerine bouillon. Tubes heated to 55° for ten minutes gave subsequent growth; those heated to 60° for ten minutes, no growth; those heated to 70° for ten minutes, no growth. The tubercle bacilli "with spores" came from an old culture in glycerin bouillon. Tubes heated to 55° for ten minutes gave subsequent growth; those heated to 60° for ten minutes gave subsequent growth; those heated to 65° for ten minutes, no growth; those heated to 70° for 10 minutes, no growth.

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[a] Martin, Hippolyte: Sur la transformation du tubercule vrai ou infectieux en corps étranger inerte sous l'influence de hautes températures et de réactifs divers. Rev. de méd., vol. 2, 1882, p. 913–927.

[b] May, F.: Ueber die Infectiosität der Milch perlsüchtiger Kühe. Arch. f. Hyg., vol. 1, 1883, p. 121.

[c] Sormani: Annali universali di med., vol. 269, 1884.

[d] Schill and Fischer: Ueber die Desinfection des Auswurfs der Phtisiker. Mittheil. a. d. k. Gesndh., vol. 2, 1884, p. 131.

[e] Voelsch: Beiträge zur Frage nach der Tenacität der Tuberkelbacillen. Ziegler & Nauwerk's Beiträge zur path. Anat. u. Phys., vol. 2, 1887.

[f] Yersin, A.: De l'action de quelques antiseptiques et de la chaleur sur le bacille de la tuberculose. Ann. de l'Inst. Pasteur, vol. 2, 1888, p. 60.

Yersin concludes that the bacillus of tuberculosis resists a temperature of 60° C. for ten minutes, and that it is remarkable that the resistance of the "spores" to the heat is not greater than that of the bacilli themselves. He made no tests upon animals.

He found, contrary to the work of Schill and Fischer, that tuberculous sputum heated to 100° for two minutes is always sterile.

Thus early in the history of these investigations the idea became prevalent that the tubercle bacillus possessed a resistance to heat considerably greater than that of other nonspore-bearing pathogens. Indeed, at this time the tubercle bacillus was regarded by many as occurring in a spore-bearing form, and some of the writers spend much time in telling whether the strains they used were spore-bearing or not, omitting more pertinent facts.

Bitter,[a] 1890, accepted the results of Yersin to the effect that the tubercle bacillus when evenly distributed through milk is killed by an exposure to 75° C. for ten minutes, and inquires if a lower temperature, desirable for practical reasons, might not be equally efficacious. He cautiously descends as far as 68° to 69°, and finds that milk to which infected sputum had been added and placed in test tubes immersed in a previously warmed water bath is rendered innocuous to guinea pigs inoculated intraperitoneally, if the exposure has lasted as much as twenty minutes. Controls injected with the unheated milk died of tuberculosis in five weeks. To be perfectly safe, the author recommends that the exposure to 68°–69° be increased to thirty minutes.

Bang,[b] 1891, states that his experiments demonstrate that heating to 85° C., and sometimes even to 80° C., kills all the tubercle bacilli; that heating for five minutes to 70° C., sometimes to 60° C., so enfeebles the virus that he believes it is no longer able to infect when fed by way of the digestive tract. Ten years later Bang was obliged to correct in part some of his early results.

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[a] Bitter, H.: Versuche über das Pasteurisiren der Milch. Zeit. f. Hyg., vol. 8, 1890, p. 240–286.

[b] Bang: Le danger supposé de la consommation du lait et de la viande sains en apparence mais provenent d'animaux atteints de la tuberculose. Trans. internat. Cong. hyg. et démog., 1890, p. 193. Also: Experimentelle Untersuchungen über tuberculöse Milch. Deut. Zeit. f. Tiermed., vol. 17, 1891, p. 1.

Many observers nowadays would regard these results as indicating an unduly high resistance of the organism and would suspect that Bang gave the tubercle bacillus a handicap in three ways: (1) In using pathological milk prone to form little clots on heating, which protect the tubercle bacilli (see Barthel and Stenström); (2) in not avoiding the formation of the protective surface pellicle (see Th. Smith, Russell and Hastings, Hesse), and (3) possibly in failing to differentiate the lesions caused by dead tubercle from those caused by living germs (Smith, Miller, Prudden, and Hodenphyl).

Bonhoff,[a] 1892, did not work with milk, but gives some data obtained with pure cultures of tubercle bacilli in liquid media, which may be accepted as contributory evidence. Young cultures, the ultimate antecedents of which are not stated, were exposed in test tubes placed in a water-bath, the temperature being observed on a thermometer placed inside the tube. Guinea pigs were inoculated intraperitoneally before and after exposure. The significant findings are, that while 50° for sixty minutes failed to kill the bacteria, 60° for twenty minutes did kill them. Exposures of less than twenty minutes were not made. The author unfortunately had some difficulty in preventing the development of lung tuberculosis in his animals. For instance, the pigs receiving the material heated to 60° for forty minutes, 70° for sixty minutes, and 80° momentarily, developed what he calls and is at considerable pains to differentiate as *inhalation* tuberculosis.

Grancher and Ledoux-Lebard,[b] 1892, appreciated the occurrence of lesions produced by dead tubercle bacilli in their inoculated animals. They showed that human tubercle bacilli are more sensitive to the action of temperatures of 50° C. than are avian tubercle bacilli. Heating the avian bacilli to 50° or 60° for fifteen minutes did not kill them. Animals inoculated died of tuberculosis. However, the organisms heated to 60° were attenuated. Heating to 70° under the same conditions noted was sufficient to destroy the power of the avian tubercle bacilli to infect laboratory animals.

They heated cultures of human tubercle bacilli suspended in distilled water and found that five minutes at 60° sufficed to attenuate the virulence. Heating to 60° for ten minutes and twenty minutes also greatly attenuated the virulence. When heated to 70° C. for one, two, five, or ten minutes the tubercle bacilli were killed.

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[a] Bonhoff: Die Einwirkung höherer Wärmegrade auf Tuberkelbacillen-Reinkulturen. Hyg. Runds., vol. 2, 1892, p. 1009.

[b] Grancher, J., and Ledoux-Lebard: Tuberculose aviaire et humaine. Arch. de méd. expér. et d'anat. pathol., vol. 4, 1892, p. 1.

Forster,[a] 1892, sealed off small glass tubes containing for the most part the milklike material squeezed from the cut surface of tubercular udders and placed these in a water bath kept at the desired temperature for various periods. They were then cooled quickly and injected intraperitoneally into guinea pigs. The presence or absence of living tubercle bacilli was determined by autopsy, by the lesions, or by carrying the suspect material over into other animals. The bacilli appeared not to be killed by three hours exposure to 55°, forty-five minutes exposure to 60°, or momentary exposure to 80°, but were killed by six hours exposure to 55° (sputum), one hour exposure to 60° and 80°, momentary exposure to 95°. These figures indicate a high degree of resistance.

It is difficult to explain the high figures obtained by Forster, especially in the light of the work of Smith, Russell and Hastings and my own.

The next year the same author,[b] reviewing the situation, emphasizes that pasteurizing apparatus is of two kinds; (1) in which the milk passes over heated surfaces and is momentarily raised to a given temperature, and (2) in which the milk is heated in kettles, bottles, etc., and may be kept for a certain time at the desired temperature. He says that the latter are the only ones applicable to milk containing tubercle bacilli, because momentary heating to temperatures insufficient to change the taste of the milk does not kill tubercle bacilli.

De Man,[c] 1893, working under Forster, obtained results long current in text-books and which have been accepted as the standards for practical work in many regulations for dairy methods. The following brief summary of early work on the subject culled by him from Galtier (1879) shows the discrepancies and inadequacies of this period—faults, however, which continue to appear in recent work:

Author.	Temperature.	Time.	Result.
Chaveau and Arloing	70°, 100°	30 minutes	No certainty of killing tubercle bacilli.
Touissaint	73°, 76°, 80°	(?)	Virulence remains.
Gerlach	100°	30 minutes	Virulence remains, but weakened.
Martin	80°, 100°	(?)	Virus withstands this heat.
Klebs			Boiled milk may cause tuberculosis.
Frerichs, Parrot, and Martin			Boiled tubercular sputum innocuous.
Vallin			Paper impregnated with tubercle bacilli loses virulence by dipping in boiling water.

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[a] Forster, J.: Ueber die Einwirkung hoher Temperaturen auf Tuberkelbacillen. Hyg. Runds., vol. 2, 1892, p. 869.

[b] Forster, J.: Ueber die Einwirkung hoher Temperaturen auf Tuberkelbacillen. Hyg. Runds., vol. 3, 1893, p. 669.

[c] De Man, C.: Über die Einwirkung von hoher Temperaturen auf Tuberkelbacillen. Arch. f. Hyg., vol. 18, 1893, p. 133.

De Man states that few, if any, of these authors recite the conditions and methods of their experiments. He used material from the substance scraped from the cut surface of tuberculous udders, and in some instances tubercular sputum. The work was done in the same manner as Forster's, q. v., and is apparently a part of or a continuation of the same series of experiments. His results may be condensed as follows:

Tubercle bacilli are killed by the following temperatures: In udder juice, 55° after four hours exposure, 60° after one hour exposure (not after forty-five minutes), 80° after five minutes exposure, 90° after two minutes exposure, 95° after one minute exposure; in sputum, 65° after fifteen minutes exposure, 70° after ten minutes exposure. Exposure for less time failed to kill. Control animals injected with unheated material became tubercular.

Smith,[a] 1899, says, in criticism of this work, that such dense suspensions can not be used to determine the thermal death point to be compared with those of other bacilli, nor can they be regarded as imitating the conditions under which tubercle bacilli occur in milk. It might be added that De Man's work at two very interesting temperatures (65° and 70°) was performed with sputum and not even udder juice.

Schroeder,[b] 1894, obtained what may be regarded as an early hint that comparatively low temperatures may be effective in killing the tubercle bacillus in milk. With most other observers at that time, he had been led to regard the tubercle bacillus as a nonspore-bearing pathogen almost unique among this class in its resistance to heat. He acted upon the advice of Yersin who, at the suggestion of Roux, attempted to make use of this property to rid milk of those other organisms which frequently produce disease or death when injected into animals. Schroeder, attempting to discover the presence of tubercle bacilli in market milk, heated the specimens to 60° for fifteen minutes before injection. Fortunately, he controlled this procedure with milk artificially inoculated with tubercle bacilli, and the failure to get tuberculosis in market milk specimens was paralleled with a similar failure in the controls. The inference was that 60° for fifteen minutes could, under some circumstances, kill tubercle bacilli.

Woodhead,[c] 1895, obtained very discordant results, which it is difficult to interpret. It is probable that they are explained, in the case of experiments conducted with milk artificially contaminated with pieces of tubercular tissue, by the fact that clumps of considerable size (too large to pass through the inoculating needle and requiring glass pipettes for inoculation) were added to the milk, and that these would in some manner protect the bacilli; and in the case of naturally tuberculous milk by the probability of protection by coagula—especially prone to occur in the concentrated pathological product—and by the pellicle which forms on the surface of heated milk.

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[a] Smith, Theobald: The thermal death point of tubercle bacilli in milk and other fluids. Journ. Exper. Med., vol. 4, 1899, p. 217.

[b] Schroeder, E. C.: Further experimental observations on the presence of tubercle bacilli in the milk of cows. U. S. Bur. An. Industry, Bull. 7, 1894, p. 75.

[c] Woodhead, G. Sims: Report of the Royal Commission appointed to inquire into the effect of food derived from tuberculous animals on human health. Parts 8, 9, 12, and 13. 1895.

Considering the inoculation experiments with both the natural and the artificial tuberculous milk, the following minimum lethal temperature exposures are found in the tables: 50° C. killed surely only after fifteen hours; 55°, after seven hours; 60°, after eight hours; 65°, after four hours;[a] 70°, after forty-five minutes; 75°, after thirty minutes; 80°, —; 85°, after two and one-half minutes; 90° did not kill.

This summary shows how Woodhead's results contradict themselves and differ from those of other observers. When the results with the naturally tuberculous milk alone are considered, the results accord more with Bang's; for example: 60° kills surely after forty-five minutes; 65°, after two and one-half minutes; 70°, after two and one-half minutes.

Marshal,[b] 1899, first sterilized milk, then added tuberculous material from cows, injected control animals with this suspension, and then exposed the milk in shallow dishes in a "pail sterilizer" placed on a water bath. Heat was applied to the water bath until the temperature in all parts of the vapor chamber was 68°. This temperature was maintained twenty minutes. The shallow layer of milk was devised to secure equal temperature throughout the milk. The controls died in a few weeks of tuberculosis, but none of the test animals killed and examined at the same time showed any signs of tuberculosis. He states that from other experience he knows that 60° for ten minutes will not kill tubercle bacilli, but does not detail the experiments upon which this conclusion is based.

Theobald Smith,[c] 1899, contributed an article to the literature of this subject which marks an era in the history of the pasteurization of milk based upon the thermal death point of the tubercle bacillus. Smith's experiments were most carefully performed. Many pitfalls and errors which some of his predecessors had fallen into were avoided and pointed out. The only possible objection to the work, which applies with equal force to all laboratory experiments, is that it was not done on a scale of practical dairy pasteurization.

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[a] No experiment between three and four hours; killed after two hours.

[b] Marshal, Charles E.: Killing the tubercle bacillus in milk. Michigan State Agricultural College experiment station, Bull. 173, 1899.

[c] Smith, Th.: The thermal death point of tubercle bacilli in milk and some other fluids. Journ. Exper. Med., vol. 4, 1899, p. 219.

He limited his investigation to the effects of 60° upon artificially infected milk and demonstrated by a series of experiments, which should be consulted in the original to appreciate their full force and value, that an exposure to 60° for fifteen minutes is enough to kill tubercle bacilli suspended in distilled water, normal salt solution, bouillon and milk, provided that in the case of milk the production of a surface pellicle is prevented. The pellicle which forms during the exposure may contain living tubercle bacilli after sixty minutes exposure at 60° C.

Smith concluded that (1) tubercle bacilli when suspended in distilled water, normal salt solution, bouillon and milk are destroyed at 60° C. in fifteen to twenty minutes (the larger number are destroyed in five to fifteen minutes); (2) when tubercle bacilli are suspended in milk the pellicle which forms during the exposure at 60° C. may contain living bacilli after sixty minutes. He also states that these experiments demonstrate that tubercle bacilli are no more resistant to heat than many other bacilli not producing spores.

Forster and De Man, had they used a homogeneous suspension in normal milk, must have arrived at similar results with their sealed tubes, where a pellicle does not form; but as Barthel and Stenström have shown, the pathological material from tuberculous udders, probably because of its abnormal reaction, is especially prone upon heating to the formation of little clumps of proteid coagula, which exert a protective influence against heat upon the tubercle bacilli.

Morgenroth,[a] 1900, apparently had not the benefit of Smith's work when he did his own experiments. He determined that 70° for ten minutes or 100° momentarily did not kill the tubercle bacilli in milk from tuberculous udders. Scum formation was not mentioned and the minutiæ of the experiments are omitted. He worked with the "thermophore," an apparatus which maintains a heat of 55° or more for several hours, and says that artificially inoculated milk is freed from living tubercle bacilli in this apparatus after three hours, two hours being insufficient.

Kobrak,[b] 1900, states that the thermophore kills tubercle bacilli in milk infected with sputum in four, but not in three, hours. The temperature ranged probably somewhat over 50° during this time.

Beck,[c] 1900, concludes from his experiments that raising milk to the boiling point is insufficient to kill the contained tubercle bacilli, but that they are surely killed by three minutes boiling. He also found that when sterilized milk infected with fine suspensions of tubercle bacilli was exposed to 70° for ten minutes, 70° for thirty minutes, and 80° for thirty minutes, tuberculosis resulted in inoculated guinea pigs.

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[a] Morgenroth: Versuche über Abtödtung von Tuberkelbacillen in Milch. Hyg. Runds., vol. 10, 1900, p. 685.

[b] Kobrak: Die Bedeutung des Milch-Thermophores für die Säuglingsernährung. Zeit. f. Hyg., 1900, vol. 34, p. 518.

[c] Beck: Experimentelle Beiträge zur Untersuchungen über die Marktmilch. Deut. Viertelj. f. off. Gesundhpfl., vol. 32, 1900, p. 430.

Galtier,[a] 1900, found that five to six minutes heating of milk to 70° C., and even 85° C., was not sufficient to destroy the tubercle bacillus when that product is richly contaminated. He prepared an emulsion from the spleens and lungs of tuberculous rabbits, which was mixed up with the milk. He concludes from his studies that milk richly contaminated by the addition of tuberculous matter is not surely sterilized at 85° for six minutes. He recommends boiling as the only safe procedure to practice.

Rabinowitsch,[b] 1900, made the statement that recent investigations at the Institut für Infektionskrankheiten in Berlin had shown that tubercle bacilli in milk are surely killed only by a temperature of 100° C. Her results recalled attention to the fact that the tubercle bacillus, like all other organisms embedded in fat, is more resistant to heat, and that this may account for the irregular results obtained by investigators.

Russell and Hastings,[c] 1900, made a valuable contribution to our knowledge of pasteurization, inasmuch as they applied the principles elucidated by Smith to work upon a practical scale. They made use of a Potts' pasteurizer, which represents that type of machine which permits of heating milk for a specified time at a specified temperature in a close chamber, thorough distribution of the heat being secured by the rotary movements of the machine. Bovine cultures of determined virulence were scraped from dog-serum slants rubbed up with a 0.6 per cent salt solution, sedimented to remove large clumps, and then the supernatant suspension was added in definite proportions to the milk. This infected milk, after control animals had been inoculated, was placed in small glass tubes closed with a cork and rubber guard, and these tubes were then heated in a water bath up to the desired temperature and then dropped into the apparatus already raised to that point. During the exposure the temperature of the milk fluctuated no more than 1 degree and that only momentarily. The only temperature employed was 60° C. After a specified time the tubes were removed and the contents injected intraperitoneally into guinea pigs. The results are clearly shown in the following tables:

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[a] Galtier, V.: Le lait tuberculeux cesse-t-il d'être dangereux après un court chauffage à 70–75 degrés? Compt. rend. des seances et mém. de la soc. de biol., vol. 2, 2d ser., 1900, p. 120.

[b] Rabinowitsch, Lydia: Deut. med. Woch., vol. 26, 1900, p. 491.

[c] Russell, H. L., and Hastings, E. G.: Thermal death point of tubercle bacilli under commercial conditions. University of Wisconsin Agricultural Experiment Station, 17th Ann. Rep., 1900.

Time exposed.	Result in each series.				
	I.	II.	III.	IV.	V.
60°, 45 minutes	— —	....	....	....	....
60°, 30 minutes	— —	....	....	....	....
60°, 20 minutes	....	— —	....	....	— —
60°, 15 minutes	— —	— —	....	....	— —
60°, 10 minutes	....	— —	— —	— —	— —
60°, 5 minutes	....	....	+ +	+ +	....
Control	+	+	+	+	+

— = No tuberculosis.
+ = Tuberculosis.
Two pigs in each experiment.

A series was then run which shows the difference in effect of heating in open vessels and in sealed or closed tubes, the latter being placed in Potts' machine.

Time exposed.	Series VI.		Series VII.	
	Potts' sealed tube.	Open bottle.	Potts' sealed tube.	Open bottle.
60°, 15 minutes	....	....	....	+ +
60°, 10 minutes	— —	+ +	— —	+ +
60°, 5 minutes	+ +	+ +	+ +	....
Control	+	+	+	....

— = No tuberculosis.
+ = Tuberculosis.
Two pigs in each experiment.

In their experiments, milk heated at 60 degrees began to show visible signs of pellicle after about twenty minutes, but the authors think that flocculent patches exist before this. For practical work they advise pasteurization at 60 degrees for twenty minutes in closed apparatus.

Herr,[a] 1901, worked with cream only. Here the problem is different from that of milk pasteurization. It is not necessary to avoid those temperatures which change the taste of the product, which is used for making butter, and hence a higher temperature and very brief exposure may be employed, permitting the use of the so-called continuous pasteurizers which operate with a great economy of time and fuel. In Herr's experiments cream was shaken up with tuberculous sputum, the mixture sealed off in tubes of 4 c. c. capacity, leaving as little air as possible in the tubes. He had previously determined how long it would take to heat the contents of such tubes to certain temperatures by dropping them into boiling water. The infected tubes were therefore placed in boiling water for the time necessary to heat to the desired temperature and then placed in a water bath maintained at this temperature. After the lapse of the desired time they were cooled rapidly and injected into guinea pigs intraperitoneally. The results are shown in the table, + and – indicating that the animals became tuberculous or not. The results were controlled by examination after the death or killing of the animals.

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[a] Herr: Das Pasteurisiren des Rahmes als Schutz gegen die Verbreitung der Tuberkelbacillen durch Butter. Zeit. f. Hyg., vol. 38, 1901, p. 182.

Temperature and time.	Result.	Temperature and time.	Result.
65°, 15 minutes	–	80°, 3 minutes	–
68°, 15 minutes	–	85°, 5 seconds	–
70°, 7 minutes	–	85°, 5 minutes	–
72°, 7 minutes	–	89°, 30 seconds	–
75°, 3 minutes	–	90°, 5 seconds	–
75°, 5 minutes	([a])	95°, 5 seconds	–
80°, 5 seconds	+		

[a] Pseudo-tubercle, Pfeiffer.

As the most practical temperature and exposure, Herr recommends 85° for two minutes for cream from which butter is to be made. This is obtained in the "continuous" form of apparatus.

Hesse,[a] 1901, corroborates Smith's findings. Sterilized milk inoculated with a culture of human tubercle bacilli several weeks old was sealed off in small tubes and these dropped into a Pfund's sterilizer previously heated. After twenty minutes exposure they were taken out and cooled and the contents injected into guinea pigs. The animals were killed after fifty-five days. Controls receiving unheated milk had high-grade generalized tuberculosis. Pigs receiving milk heated at 57° for twenty minutes had less severe lesions; those receiving milk heated at 58° for twenty minutes had still severe lesions, and one receiving milk heated at 60° for twenty minutes was entirely sound, the other having a small translucent nodule in the diaphragm, in which no tubercle bacilli were found.

The experiments corroborate Smith's findings. The gradation of severity of lesions inversely with the increased temperature is interesting.

Levy and Bruns,[b] 1901, showed that a temperature varying between 65° and 70° C. and operating for fifteen to twenty-five minutes effectually killed bovine and human tubercle bacilli in milk, when 1 liter of the artificially infected milk was exposed in a d'Arsonval thermostat—i. e., under conditions where a pellicle would not be formed. The work is interesting especially because a liter of milk was used in the experiments. It is to be regretted that they did not experiment with lower temperatures.

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[a] Hesse: Ueber die Abtödtung der Tuberkelbacillen in 60° C. warmer Milch. Zeit. Tiermed., 1901, vol. 5, p. 5–6.

[b] Levy, E., and Bruns, Hugo: Ueber die Abtödtung der Tuberkelbacillen in der Milch durch Einwirkung von Temperaturen unter 100°. Hyg. Runds., vol. 11, 1901, p. 669.

Barthel and Stenström,[a] 1901, report some preliminary work which must be interpreted as showing the influence of coagula in protecting the tubercle bacilli from the action of heat. This clot formation was probably due to the abnormal reaction of the pathological fluid from tuberculous udders employed. Animals injected with this material became tuberculous after it had been heated to 75° for fifteen minutes and 80° for ten minutes. Temperatures and exposures sufficient to kill the tubercle bacilli under the conditions of the experiments were not employed. In this connection the subsequent work of these authors should be consulted (vide infra).

Bang,[b] 1902, reported a large number of experiments which give results at variance with those of this author's former investigations. To avoid the formation of the surface pellicle and foam, in some of his work he exposed the milk in closed metallic boxes containing a thermometer immersed in a water bath and kept constantly in motion during the exposure to insure an equal distribution of the heat. As in former work, he used rabbits to test the virulence of exposed material by the intraperitoneal method. He experimented with pure cultures of the bacillus, noting whether growth was delayed or inhibited by exposure to heat, and further determined the effect of feeding the heated milk to rabbits and swine. The essential features of his results (under conditions which prevent the formation of scum) are as follows:

Temperature and exposure.	Result.
60°, 15 minutes	Prevented tuberculosis in 2 animals, but 2 others became slightly tuberculous.
65°, 1 to 15 minutes	Effectually prevented tuberculosis.
70°, 75°, 80°, and 85°, momentarily	Do.

Tuberculous milk from diseased udders was used in some of these experiments.

Rabbits receiving one 30 c. c. feeding of milk exposed for two minutes to 60°, 65°, 70°, 75°, 80°, and 85° remained free from tubercle, although controls fed with raw milk became tuberculous.

Swine fed with 150 c. c. each of tuberculous milk heated for half a minute at 60° became tuberculous, while those receiving milk heated to 70°, 75°, and 80° for half a minute remained sound.

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[a] Barthel, Chr., and Stenström, O.: Beitrag zur Frage des Einflusses hoher Temperaturen auf Tuberkelbacillen. Cent. f. Bakt., vol. 30, 1901, p. 429.

[b] Bang: Ueber die Abtödtung der Tuberkelbacillen bei Wärme. Zeit. f. Tiermed., vol. 6, 1902, p. 81.

From these results Bang concludes that while heating at 60° for fifteen minutes does not prevent peritoneal infection it weakens the bacteria so that they are innocuous by way of the alimentary tract.

Concerning his work with cultures, it is interesting to note that glycerin-bouillon tubes sowed with particles of growth from a young culture failed to develop any growth after exposure to 60° and 65° for five minutes, although unheated controls grew well.

Bang did not report results at 60° for twenty minutes.

Tjaden,[a] 1903, obtained very high thermal death points for the tubercle bacillus and concludes that 85° for one to two minutes is necessary to kill that organism. Bang attributes this high resistance to the coagula which formed during some of the experiments.

Rullmann,[b] 1903, in a preliminary note, says that he can not agree with those observers who state that 60° for fifteen or twenty minutes kills the tubercle bacilli in milk. He found that milk infected with tuberculous sputum exposed to 65° for half an hour "with all precautions" still caused tuberculosis when injected intraperitoneally into guinea pigs. He promises a full report, which, however, we have not been able to obtain.

Barthel and Stenström,[c] 1904, continue their experiments relative to the effect of the reaction of milk upon the resistance to heat of the contained tubercle bacilli. They divided into two portions some tuberculous milk; one portion was rendered abnormally acid, another neutral in reaction. The neutral milk withstood 90° without coagulation; the acid milk coagulated at 60°.

Results with 80°: One minute exposure killed in uncoagulated milk, but not in coagulated, i. e., acid milk.

At 85°: Momentary exposure failed to kill in either. One minute exposure killed in uncoagulated but not in coagulated milk.

While these experiments are very limited in scope, they at least suggest a source of error in the work of those who used the secretion of tubercular udders in their experiments. Such material is prone to be abnormal in reaction, and protective clots may have been formed.

Sydney Phillips,[d] 1904, mentions merely that the results obtained by Russell and Hastings[e] were confirmed by Macfadyean of the Lister Institute.

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[a] Tjaden: Abtödtung der pathogenen Keime in der Molkereimilch durch Erhitzung ohne Schädigung der Milch und Milchproducte. Deut. Med. Woch., 1903, p. 976.

[b] Rullman: Ueber die Abtödtung von Tuberkelbacillen in erhitzer Milch. Münch. med. Woch., 1903, vol. 31, p. 1342.

[c] Barthel, Chr., and Stenström, O.: Weitere Beiträge zur Frage des Einflusses hoher Temperaturen auf Tuberkelbacillen in der Milch. Cent. f. Bakt., vol. 37, 1904, p. 459.

[d] Phillips, Sydney: The milk supply of a hospital; American method of sterilization. The Hospital, July 16, 1904.

[e] Russell and Hastings. University of Wisconsin Agricultural Experiment Station, 17th Ann. Rep., 1900.

Russell and Hastings,[a] 1904, had worked previously from the standpoint of a comparatively low temperature, 60° C. They now worked from the standpoint of a short exposure, such, for instance, as is available in a continuous-action pasteurizing machine, to determine how low a temperature could be safely used with such necessarily short exposures. Milk infected with human and bovine cultures of known origin and virulence was exposed in sealed glass tubes placed in a water bath. It took three-fourths of a minute for the contained milk to reach the temperature of the outside water. Virulence was determined upon guinea pigs by the intraperitoneal method.

Their results show that material exposed to 160° F. (71.1° C.) for one minute was freed of living tubercle bacilli. This applied to a very virulent bovine strain as well as to less virulent bovine and human strains. The authors conclude that 71.1° C. or more for a period of one minute suffices to destroy the virulence of bovine tubercle cultures so that the disease is not produced in guinea pigs inoculated with amounts of the cultures ranging from 2 to 5 milligrams. As definite amounts of the culture had been added to the milk, the dosage of each animal could be approximated. It is fair to note that the virulent bovine strain produced lesions in animals receiving the heated milk, which, however, were justly regarded by the authors as being due to dead bacteria, since animals inoculated with this material failed to develop tuberculosis. The authors do not unreservedly recommend this temperature and exposure for routine practical pasteurization without further investigation under all conditions.

Schwellengroebel,[b] 1904, shows that when milk is pasteurized in bottles the tubercle bacilli, if present, may be protected against the heat by (1) the rough or cracked surface of the rubber washers used by some dairymen in closing the bottles, (2) the dried layer of milk in improperly cleansed bottles, (3) the pellicle formed on the surface of heated milk, and (4) the foam formed in the same way. The remedies are, of course, sterilization of bottles, washers, etc., and heating under conditions which prevent the formation of foam and pellicle.

Zelenski,[c] 1906, is a recent advocate of higher temperatures in the pasteurization of milk. He finds that 76° for ten, fifteen, or twenty minutes is insufficient to kill the bacilli.

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[a] Russell, H. L., and Hastings, E. G.: Effects of short periods of exposure to heat on tubercle bacilli in milk. University of Wisconsin Agricultural Experiment Station, 21st Ann. Rep., 1904, p. 178.

[b] Schwellengröbel, M.: Ueber das Pasteurisiren der Milch. Cent. f. Bakt., Abt. 2, vol. 12, 1904, p. 440.

[c] Zelenski: Zur Frage der Pasteurisation der Säuglingsmilch. Jahrb. f. Kinderheilkunde, 1906, vol. 63, 3, p. 288.

It is difficult, if not impossible, to briefly summarize the above work. The following table necessarily leaves out many factors:

TABLE NO. 12.—*Showing the thermal death point of the tubercle bacillus as found by various investigators.*

Investigator.	Killed at—	Not killed at—
Martin, 1882		80°.
May, 1883	By cooking	
Sormani, 1884	Boiling, 5 minutes	90° for 10 minutes.
Schill and Fisher, 1884		100°.
Voelsch, 1887		100°, boiling twice.
Yersin, 1888	60°, 10 minutes (−spores) 60°, 10 minutes (+spores)	
Bitter, 1890	68°, 20 minutes	
Bang, 1891	70°, 5 minutes (enfeebles) 60°, 5 minutes (sometimes enfeebles) 80° (sometimes kills) 85° (always kills)	
Bonhoff, 1892	60°, 20 minutes	50°, 60 minutes.
Grancher and Ledeux-Lebard, 1892	60°, 5 minutes (attenuates) 70°, 1 minute (kills)	
Forster, 1892	60°, 6 hours 95°, momentary	55°, 3 hours. 60°, 45 minutes. 80°, momentary.
De Man, 1893	55°, 4 hours 60°, 1 hour	60°, 45 minutes.
Schroeder, 1894	60°, 15 minutes	
Woodhead, 1895	50°, 15 hours 60°, 8 hours 60°, 45 minutes 70°, 45 minutes 70°, 2½ minutes	90° (results contradictory).
Marshall, 1899	68°, 20 minutes	60°, 10 minutes.
Th. Smith, 1899	60°, 15 to 20 minutes	
Morgenroth, 1900	55°, 3 hours	70°, 10 minutes. 100°, momentary.
Kobrak, 1900	50°, 4 hours	
Beck, 1900	100°, 3 hours	100° 80°, 30 minutes.
Galtier, 1900		85°, 6 minutes.
Russell and Hastings, 1900	60°, 20 minutes	
Herr, 1901	65°, 15 minutes	80°, 5 seconds.
Hesse, 1901	60°, 20 minutes	
Levy and Bruns, 1901	65°, 15 minutes	
Barthel and Stenström, 1901		70°, 15 minutes.
Bang, 1902		60°, 15 minutes.
Tjaden, 1903	85°, 1 to 2 minutes	
Rullmann, 1903	65°, 30 minutes	60°, 20 minutes.
Barthel and Stenström, 1904	80°, 1 minute (uncoagulated)	80°, 1 minute (coagulated).
Russell and Hastings, 1904	71°, 1 minute	
Zelenski, 1906		76°, 20 minutes.
Rosenau, 1907	60°, 20 minutes	

The above tabular statement shows that my results agree with the work of Yersin, Bonhoff, Schroeder, Th. Smith, Russell and Hastings, and Hesse in that 60° for twenty minutes is sufficient to kill the tubercle bacillus.

BACILLUS TYPHOSUS.

REVIEW OF THE WORK OF OTHERS.

Sternberg,[a] 1888, found that the temperature of 56° C. (138.8° F.) for ten minutes killed the Bacillus *typhi abdominalis.* Sternberg adopted ten minutes as the standard time of exposure for his experimental work upon the thermal death point of a long list of micro-organisms. He took pains to insure uniform temperature by personal attention and frequent stirring of the water bath with a glass rod. The micro-organisms were first drawn into hermetically sealed capillary tubes with expanded extremities. The tubes were immersed in a water bath, maintained at the desired temperature, for the standard time, namely, ten minutes. No attempt was made to fix the thermal death point within narrower limits than 2° C.

Pfuhl,[b] 1888, placed a number of test tubes containing a bouillon culture of typhoid bacilli with spores (?) in water baths at 50° and 60° C. for ten, twenty, thirty, and fifty minutes. All the 50° cultures retained life and motility. In the 60° bath only those exposed ten minutes regularly showed movement and growth.

Van Geuns,[c] 1889, determined the effect upon bacteria of momentary heating to temperatures varying from 50° to 80° C. and immediate cooling. He used four methods:

Method 1: Stock-broth culture of the organism was introduced into 10 per cent gelatin tubes with a platinum loop. Some of these inoculated tubes were incubated as controls and the others placed in a water bath kept at a constant temperature. The control with thermometer in a gelatin tube showed that it took ninety seconds to reach 80° C. and that it took about the same time to cool the tube in tap water to 12° C. After heating, the gelatin tubes were incubated at 20°. This was the first method used by Van Geuns for a study of the common organisms in milk.

For special purposes he adopted one of the following methods:

Method 2: Capillary pipettes were filled with broth cultures. The capillary tube was inserted into a cork along with a thermometer and both immersed into a test tube of water, which in turn rested in a water bath at boiling temperature. As soon as the required temperature in the tube was reached it was removed from the bath and cooled. The capillary tube was then broken, and, with a needle, stabs were made in gelatin.

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[a] Sternberg, Manual of Bacteriology, 1892, p. 146, and Report of the Committee on Disinfectants of the American Public Health Association. 1888, pp. 136 and 152 (p. 140).

[b] Pfuhl: Zur Sporenbildung der Typhusbacillen. Cent. f. Bakt., vol. 4, 1888, p.771.

[c] Van Geuns: Über das Pasteurisiren von Bakterien. Ein Beiträgé zur Biologie der Microorganismen. Arch. f. Hyg., vol. 9, 1889, p. 369.

Method 3: This is a modification of method No. 1. The test tube, containing broth or water, with a thermometer and capillary tube, is put into a water bath kept at temperature of the tube tested, or 3 to 5 degrees higher.

Method 4: Capillary tubes and the thermometer placed in a small Erlenmyer flask, which can be well shaken. The fluid in the flask was accurately maintained at a constant temperature by placing the flask in a small water bath, which, in turn, was placed in a larger one and a thermoregulator used to control the temperature of the outer bath.

Van Geuns concluded that the thermal death points based on the above experiments were as follows:

Time required to reach temperature stated.	Cholera. (° C.)	Typhoid. (° C.)
1 minute	59	60
5 minutes	54	56

Lazarus,[a] 1890, used 20 liters of raw market milk divided into small flasks; these he inoculated with cholera and typhoid. This infected milk was then run through a small Thiel's continuous pasteurizer at the rate of 1 liter per forty seconds. The temperatures were made to vary from 60° to 96° C. in the different experiments. At intervals of one to two minutes samples of one-half cubic centimeter were taken and plated on fluid gelatin. At 70° C. living typhoid and cholera were found, but none at 75° C. These results are more a test of the efficiency of this particular pasteurizer than an accurate determination of the thermal death points of the micro-organisms used.

Janowski,[b] 1890, inoculated typhoid bacilli from a four-day potato growth into gelatin after it reached the temperature to be tested. At the end of five or ten minutes roll tubes were made. He found those exposed 57° for five minutes always remained sterile. Sometimes he got a growth at 56° for five minutes, and once at 56° for ten minutes. The bacilli were not put into the gelatin until it reached the temperature to be tested, and the work therefore does not exactly imitate the conditions of pasteurization.

Neisser,[c] 1895, used the same methods as von Geuns. The temperature was taken from a control tube and the time counted from the moment the temperature reached the desired degree. Some typhoid bacilli resisted three minutes' heating at 60° to 61.75° C., but fifteen minutes' heating at this temperature killed all.

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[a] Lazarus, A.: Die gebräuchlicheren Mittel zur Conservirung der Milch. Zeit. f. Hyg., vol. 8, 1890, p. 235.

[b] Janowski: Zur Biologie der Typhusbacillen. Cent. f. Bakt., vol. 8, 1890, p. 167.

[c] Neisser, Max: Dampf-Desinfection und Sterilisation von Brunnen und Bohrlöchern. Zeit. f. Hyg., vol. 20, 1905, p. 308.

Hesse,[a] 1900, obtained sterile milk directly from the cow by the use of special precautions in test tubes, and inoculated these tubes with cultures of typhoid, cholera, diphtheria, and plague. They were heated to 60° C. for fifteen to twenty minutes in a water bath and plated. The plates remained sterile. Controls gave a good growth. No record is given of the effect of lower temperatures or shorter times. The possible effects of the "germicidal" power of the raw milk was not considered.

Bassenge,[b] 1903, made about fifty-two determinations of the effect of various temperatures upon typhoid in raw milk. He used large quantities of raw milk—one-half to 1½ liters—to which had been added a broth culture tube of typhoid. The work was done in iron, porcelain, and enamel-ware vessels, over gas and coal flames of varying intensity. The milk was raised from room temperature to the desired point. Bassenge found that when milk was raised to a temperature of 60° C. in from five to ten minutes and plates made on Conradi-Drigalski medium no typhoid was found. When, however, the milk was raised to 60° C. in three minutes, typhoid colonies were present. He does not indicate whether the degrees reported were top or bottom temperatures. His figures varied slightly, depending upon the composition of the vessel in which the milk was heated.

Friedel, Kutscher, and Meinicke,[c] 1904, working in Koch's laboratory under Kolle's direction, found that the typhoid, paratyphoid, and enteritidis group of bacilli have the same resistance to heat. They were all killed without exception when the temperature of the milk reached 59° C., provided ten minutes elapsed from the beginning of the heating until this temperature was reached.

These experiments were made by previously heating one-half to 1 liter of milk to 100° in an Erlenmeyer flask and after this cooled inoculating it with 10 c. c. of a fresh twenty-four hour bouillon culture. The flasks were then set in a large kettle of hot water and precautions taken to obtain an equable distribution of heat. It required ten minutes to heat this quantity of milk from 15° to 60° C. At intervals of one minute 5 c. c. were taken with a sterile pipette and added to 50 c. c. of bouillon, incubated twenty-four hours, and then plated upon agar.

A review of the literature upon the viability of the typhoid bacillus, with especial reference to its thermal death point, will be found in Hyg. Rundschau, vol. 14, 1904, p. 353, by Ina Rosquit, and Zeit. f. Hyg., vol. 20, 1895, p. 308, by Max Neisser.

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[a] Hesse, W.: Ueber das Verhalten pathogenen Mikroorganismen in pasteurisirter Milch. Zeit. f. Hyg., vol. 34, 1900, p. 346.

[b] Bassenge, R.: Ueber das Verhalten der Typhusbazillen in der Milch und deren Produkten. Deut. med. Woch., vol. 29, 1903, p. 264.

[c] Friedel, Kutscher, and Meinicke: Die Widerstandfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 13, 1904–5, p. 324.

ORIGINAL EXPERIMENTS.

In the following work I used nine different strains of typhoid bacilli. The results were as follows:

Experiment No. 1.

B. typhosus.

Forty-eight hours' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	Loop of milk culture in bouillon.
"Dawson" culture; reaction, 0.216 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	([a])	Do.
"P. I." culture; reaction, 0.207 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	([a])	Growth.[b]
"P. I." culture; reaction, 0.198 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	([a])	Do.
"McF." culture; reaction, 0.225 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.[c]
4 minutes after immersion	60	No growth.
5 minutes after immersion	([a])	Do.
"Smith" culture; reaction, 0.198 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	([a])	Do.
"Hay" culture; reaction, 0.234 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
4 minutes after immersion	60	No growth.
5 minutes after immersion	([a])	Do.
"Stock" culture; reaction, 0.171 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	([a])	Do.

[a] One minute at 60°.

[b] Contaminated.

[c] Delayed.

Experiment No. 1—Continued.

Milk culture.	Temperature (°C.).	Loop of milk culture in boullion.
"Chicago" culture; reaction, 0.225 per cent acid:		
At start	29	Growth.
1½ minutes after immersion	55	Do.
2¼ minutes after immersion	58	No growth.
4 minutes after immersion	60	Do.
5 minutes after immersion	(a)	Do.

a One minute at 60°.

Experiment No. 2.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in sterilized milk. Reaction, 0.28 per cent acid.

The milk at 31.5° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	31.5	Growth	70,600
1½ minutes after immersion	55	do	25,000
1¾ minutes after immersion	56		
1⅘ minutes after immersion	57	Growth	10,140
2¼ minutes after immersion	58	do	1,780
2½ minutes after immersion	59	do	121
3¾ minutes after immersion	60	No growth	1
4¾ minutes after immersion	1 minute at 60	do	None.
5¾ minutes after immersion	2 minutes at 60	do	None.
5¾ minutes after immersion	3 minutes at 60	do	None.
6¾ minutes after immersion	4 minutes at 60	do	None.
7¾ minutes after immersion	5 minutes at 60	do	None.

Experiment No. 3.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in sterilized milk. Reaction, 0.198 per cent acid.

Milk at 29° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	29	Growth	(a)
1½ minutes after immersion	55	do	24
2¼ minutes after immersion	58	No growth	Lost.
4 minutes after immersion	60	do	None.
5 minutes after immersion	1 minute at 60	do	None.

a Innumerable.

EXPERIMENT NO. 4.

B. typhosus.

Culture, "Hopkins;" twenty-four hours' growth in sterilized milk. Reaction, 0.27 per cent acid.

The milk at 27° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	27	Growth	[a] 40,000
1 minute after immersion	50	do	[a] 40,800
1½ minutes after immersion	55	do	[a] 40,600
2¼ minutes after immersion	58	do	2,400
2¾ minutes after immersion	59	do	360
4 minutes after immersion	60	do	12
6 minutes after immersion	2 minutes at 60	No growth	None.
8 minutes after immersion	4 minutes at 60	do	None.
10 minutes after immersion	6 minutes at 60	do	None.
12 minutes after immersion	8 minutes at 60	do	None.
14 minutes after immersion	10 minutes at 60	do	None.

[a] About.

EXPERIMENT NO. 5.

B. typhosus.

Twenty-four hours' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
"Stock" culture; reaction, 0.162 per cent acid:		
At start	28	Growth.
2½ minutes after immersion	58	No growth.
3 minutes after immersion	59	Do.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	Do.
6 minutes after immersion	2 minutes at 60	Do.
"Hay" culture; reaction, 0.126 per cent acid:		
At start	28	Growth.
2½ minutes after immersion	58	No growth.
3 minutes after immersion	59	Do.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	Do.
6 minutes after immersion	2 minutes at 60	Do.

Experiment No. 6.

B. typhosus.

Culture, "Hay;" forty-eight hours' growth in sterilized milk. Reaction, 0.162 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	Do.
4¼ minutes after immersion	1 minute at 60	No growth.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

Experiment No. 7.

B. typhosus.

Culture, "Chicago;" forty-eight hours' growth in sterilized milk. Reaction, 0.171 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2⅓ minutes after immersion	58	Do.
2¾ minutes after immersion	60	Do.
3¾ minutes after immersion	1 minute at 60	No growth.
4¾ minutes after immersion	2 minutes at 60	Do.
5¾ minutes after immersion	3 minutes at 60	Do.
6¾ minutes after immersion	4 minutes at 60	Do.
7¾ minutes after immersion	5 minutes at 60	Do.

Experiment No. 8.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1¾ minutes after immersion	55	Do.
2⅓ minutes after immersion	58	Do.
2¾ minutes after immersion	60	Do.
3¾ minutes after immersion	1 minute at 60	Do.

Experiment No. 8—Continued.

Milk culture.	Temperature (°C.).	⅓ c. c. milk culture in bouillon.
4¾ minutes after immersion	2 minutes at 60	No growth.
5¾ minutes after immersion	3 minutes at 60	Do.
6¾ minutes after immersion	4 minutes at 60	Do.
7¾ minutes after immersion	5 minutes at 60	Do.

Experiment No. 9.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1⅓ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	No growth.
4¼ minutes after immersion	1 minute at 60	Do.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

Experiment No. 10.

B. typhosus.

Culture, "Smith;" forty-eight hours' growth in sterilized milk. Reaction, 0.162 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1⅓ minutes after immersion	50	Do.
1⅓ minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¼ minutes after immersion	60	Do.
4¼ minutes after immersion	1 minute at 60	No growth.
5¼ minutes after immersion	2 minutes at 60	Do.
6¼ minutes after immersion	3 minutes at 60	Do.
7¼ minutes after immersion	4 minutes at 60	Do.
8¼ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT NO. 11.

B. typhosus.

Culture, "Hopkins;" forty-eight hours' growth in whole milk that had been sterilized by the fractional process three days. Reaction, 0.252 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2¼ minutes after immersion	57	Do.
3 minutes after immersion	59	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	No growth.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	Do.
14¼ minutes after immersion	10 minutes at 60	Do.

EXPERIMENT NO. 12.

B. typhosus.

Culture, "P. I.;" forty-eight hours' growth in whole milk that had been sterilized by the fractional process three days. Reaction, 0.288 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.)	1 c. c. milk culture in bouillon.
At start	21	Growth.
1½ minutes after immersion	55	Do.
2 minutes after immersion	57	Do.
2½ minutes after immersion	59	Do.
3⅔ minutes after immersion	60	Do.
4⅔ minutes after immersion	1 minute at 60	No growth.
5⅔ minutes after immersion	2 minutes at 60	Do.
6⅔ minutes after immersion	3 minutes at 60	Do.
8⅔ minutes after immersion	5 minutes at 60	Do.
13⅔ minutes after immersion	10 minutes at 60	Do.

Conclusion.—The evidence is plain that milk heated to 60° C. and maintained at that point for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C. and few survive to 60° C.

BACILLUS DIPHTHERIÆ.

REVIEW OF THE WORK OF OTHERS.

Welch and Abbott,[a] 1891, tested the thermal death point of the diphtheria bacillus upon bouillon cultures and suspensions, in sterlized salt solution, in capillary glass tubes, and found that the bacillus is killed at a temperature of 58° C. in ten minutes. Details not stated.

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[a] Welch and Abbott: The etiology of diphtheria. Johns Hopkins Hospital Bull., vol. 2, 1892.

Sternberg,[a] 1892, quotes Löffler to the effect that the thermal death point of the bacillus of diphtheria is 60° C.

ORIGINAL EXPERIMENTS.

In my work I used Park's bacillus No. 8, which is a strong toxine producer and virulent for laboratory animals. My results follow:

Experiment No. 13.

B. diphtheriæ.

Culture, "Park, No. 8;" twenty-four hours growth in sterilized milk. Reaction of milk, 0.108 per cent acid.

The milk at 32° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in glycerin agar.
At start	32	Growth	1,240
2 minutes after immersion	50	No growth	None.
2½ minutes after immersion	57	do	None.
3¾ minutes after immersion	59	do	None.
4½ minutes after immersion	60	do	None.
5½ minutes after immersion	1 minute at 60	do	None.

Experiment No. 14.

B. diphtheriæ.

Culture, "Park, No. 8;" twenty-four hours growth in sterilized milk. Reaction of milk, 0.108 per cent acid.

The milk at 31.5° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in glycerin agar.
At start	31.5	Growth	1,800
1½ minutes after immersion	55	No growth	None.
2 minutes after immersion	57	do	None.
2½ minutes after immersion	59	do	None.
3 minutes after immersion	60	do	None.
4 minutes after immersion	1 minute at 60	do	None.

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[a] Sternberg: Manual of bacteriology, 1892, p. 148; also, American Public Health Association, committee on disinfectants, p. 152.

EXPERIMENT NO. 15.

B. diphtheriæ.

Culture, "Park"; forty-eight hours' growth in sterilized milk. Reaction, 0.153 per cent acid.

The milk at 28° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
At start	28	Growth	3,150
1⅓ minutes after immersion	45	do	1,890
1½ minutes after immersion	48	do.[a]	[b]19
1¾ minutes after immersion	50	do.[a]	26
2 minutes after immersion	51	No growth	None.
2¼ minutes after immersion	52	do	None.
2⅓ minutes after immersion	53	do	None.
2¾ minutes after immersion	54	do	None.
3½ minutes after immersion	55	do	None.

[a] Delayed. [b] Contaminated.

EXPERIMENT NO. 16.

B. diphtheriæ.

Culture, "Park, No. 8;" fifty-two hours' growth in sterilized milk.

Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Three test-tube cultures tested by planting a loop over into bouillon.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.		
		Reaction, 0.171 per cent acid.	Reaction, 0.135 per cent acid.	Reaction, 0.135 per cent acid.
At start	28.5	Growth	Growth	Growth.
1⅓ minutes after immersion	50	No growth		
2½ minutes after immersion	55	do	No growth	No growth.
2⅔ minutes after immersion	58	do	do	Do.
4⅓ minutes after immersion	60	do	do	Do.
5⅓ minutes after immersion	(a)	do	do	Do.

[a] One minute at 60°.

EXPERIMENT NO. 17.

B. diphtheriæ.

Culture, "Park, No. 8;" forty-eight hours' growth in sterilized milk. Reaction of milk, 0.162 per cent acid.

The milk at 25° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	25	Growth.
1½ minutes after immersion	45	Do.
2 minutes after immersion	50	No growth.
2¼ minutes after immersion	51	Do.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53	Do.
3 minutes after immersion	54	Do.
3¾ minutes after immersion	55	Do.
4¾ minutes after immersion	1 minute at 55	Do.
5¾ minutes after immersion	2 minutes at 55	Do.
6¾ minutes after immersion	3 minutes at 55	Do.

EXPERIMENT NO. 18.

B. diphtheriæ.

Culture, "Park, No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.135 per cent acid.

The milk at 30° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.	Loop of milk culture in in bullion.
At start	30	Growth	Growth.
1⅓ minutes after immersion	45	do	Do.
1½ minutes after immersion	48	do	Growth, delayed.
1¾ minutes after immersion	50	do	Growth, small, delayed.
2 minutes after immersion	51	do	Growth, delayed.
2⅓ minutes after immersion	52	do	Growth, small, delayed.
2¾ minutes after immersion	53	do	No growth.
4 minutes after immersion	54	Growth, delayed	Do.
5¾ minutes after immersion	55	do	Do.

This experiment demonstrates the different results obtained by carrying over large and small quantities of the test fluid.

Experiment No. 19.

B. diphtheriae.

Culture, "Park;" forty-eight hours' growth in sterilized milk. Reaction, 0.09 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
1¾ minutes after immersion	53	Do.
2¼ minutes after immersion	55	Do.
2½ minutes after immersion	56	Do.
2¾ minutes after immersion	58	Growth, small.
4 minutes after immersion	60	Do.
5 minutes after immersion	1 minute at 60	No growth.
6 minutes after immersion	2 minutes at 60	Do.

Experiment No. 20.

B. diphtheriae.

Culture, "Park. No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	0.5 c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	No growth.
3 minutes after immersion	58	Do.
3½ minutes after immersion	59	Do.
4¾ minutes after immersion	60	Do.
5¾ minutes after immersion	1 minute at 60	Do.
6¾ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT No. 21.

B. diphtheriae.

Culture, "Park, No. 8;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	0.5 c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1¾ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
2¾ minutes after immersion	57	No growth.
3¼ minutes after immersion	58	Do.
4 minutes after immersion	59	Do.
5½ minutes after immersion	60	Do.
6½ minutes after immersion	60.4	Do.
7½ minutes after immersion	60.5	Do.

EXPERIMENT No. 22.

B. diphtheriae.

Culture, "Park, No. 8;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.216 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2½ minutes after immersion	55	No growth.
2¾ minutes after immersion	57	Do.
3¾ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

Conclusion.—The diphtheria bacilli succumb at comparatively low temperatures. Oftentimes they fail to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

On account of its acid reaction milk is not a suitable medium for cholera.

Van Geuns,[a] 1889, showed that 59° C. for one minute, or 54° for five minutes, was ample to kill Koch's comma bacillus.

Sternberg,[b] 1888, found that a temperature of 52° C. (125.6° F.) was sufficient to kill the *Spirillum choleræ Asiaticæ*. For details of Sternberg's technique see page 48.

Kitasato,[c] 1889, tested the thermal death point of the cholera bacillus in gelatin and found that it required 60° C. for ten minutes to kill this organism with certainty, although sometimes it dies at 50° for fifteen minutes or 55° for ten minutes. Kitasato used eight different cultures and made a number of experiments, the results of which are brought together in the following table:

	I.	III.	VI.	VIII.	IX.	X.	XII.	XIV.
50° C., 5 minutes	+	+	+	+	+	+	+	+
50° C., 10 minutes	+	+	+	+	+	+	+	+
50° C., 15 minutes	+	+	+	+	–	+	–	–
55° C., 5 minutes	+	+	+	+	+	+	+	+
55° C., 10 minutes	+	+	+	+	–	+	–	–
55° C., 15 minutes	–	+	+	–	–	–	–	–
60° C., 5 minutes	–	+	+	–	–	–	–	–
60° C., 10 minutes	–	–	–	–	–	–	–	–
65° C., 5 minutes	–	–	–	–	–	–	–	–

The method used by Kitasato was first to transfer a platinum loop of the culture into a test tube containing gelatin which had previously been melted. This tube was then introduced into a water bath which had been brought up to the temperature required. After the time stated, the gelatin tubes were rolled and incubated at 20° to 22° C. Kitasato found no difference between old and young cultures of cholera so far as their resistance to heat was concerned, and further concluded that no material difference was evident so far as the resistance of cholera cultures are concerned against temperatures of 50° to 60°.

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[a] Van Geuns: Über des Pasteurisiren von Bakterien. Ein Beiträg zur Biologie der Microorganismen. Arch. f. Hyg., vol. 9, 1889, p. 369.

[b] Sternberg: Manual of Bacteriology, 1892, p. 146, and Report of the Committee on Disinfectants of the American Public Health Association, pp. 136, 152.

[c] Kitasato, S.: Die Widerstandsfähigkeit der Cholerabacterien gegen das Eintrocknen und gegen Hitze. Zeit. f. Hyg., vol. 5, 1889, p. 135.

Friedel, Kutscher, and Meinicke,[a] 1904, found that the cholera vibrio was not killed when the temperature reached 58°, but was dead as soon as 60° was reached. It required seven to eight minutes for the milk to reach 60°.

These experiments with the cholera organism were conducted with and without the enrichment methods. The enrichment methods consist of first planting a milk culture in peptone water, incubating twenty-four hours, and then plating into litmus-lactose-agar. With the enrichment method it was found that cholera withstood sixty minutes at 50°, but only twenty-five minutes without the enrichment method. At 53° it withstood forty minutes with and fifteen minutes without the enrichment method.

ORIGINAL EXPERIMENTS.

In my work I used three different cultures with the following results:

Experiment No. 23.

B. choleræ.

Forty-eight hours' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
"Manila;" reaction, 0.180 per cent acid:			
At start	28	Growth	450,000
1½ minutes after immersion	45	....do	3,750
1⅔ minutes after immersion	48	No growth	[b] 10
1¾ minutes after immersion	50	....do	None.
2 minutes after immersion	51	....do	None.
2¼ minutes after immersion	52	....do	None.
2¾ minutes after immersion	53	....do	None.
3 minutes after immersion	54	....do	None.
3¾ minutes after immersion	55	....do	None.
"Hamburg;" reaction, 0.229 per cent acid:			
At start	28	Growth	87,500
1½ minutes after immersion	45	....do	144
1⅔ minutes after immersion	48	No growth	[b] 4
1¾ minutes after immersion	50	....do	None.
2 minutes after immersion	51	....do	None.
2¼ minutes after immersion	52	....do	None.
2¾ minutes after immersion	53	....do	None.
3 minutes after immersion	54	....do	None.
3¾ minutes after immersion	55	....do	None.

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[a] Friedel, Kutscher, and Meinicke: Die Widerstandsfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 13, 1904–05, p. 324.

[b] Contaminated.

EXPERIMENT No. 24.

B. choleræ.

Three days' growth in sterilized milk. Milk at 28° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.	Number of colonies per loop in agar.
"Manila;" reaction, 0.219 per cent acid:			
At start	28	Growth	(a)
1¼ minutes after immersion	50	(Lost	
2 minutes after immersion	55	No growth	
3 minutes after immersion	58	do	None.
5½ minutes after immersion	60	do	None.
"Paris;" reaction, 0.189 per cent acid:			
At start	28	Growth	(a)
1¼ minutes after immersion	50	No growth	
2 minutes after immersion	55	do	
3 minutes after immersion	58	do	None.
5½ minutes after immersion	60	do	None.

[a] Innumerable.

EXPERIMENT No. 25.

B. choleræ.

Culture, "Hamburg;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.216 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	(a)
1⅛ minutes after immersion	45	41
1⅜ minutes after immersion	48	None.
1¾ minutes after immersion	50	None.
2 minutes after immersion	51	None.
2¼ minutes after immersion	52	None.
2½ minutes after immersion	53	None.
3 minutes after immersion	54	None.
4 minutes after immersion	55	None.

[a] Innumerable.

EXPERIMENT NO. 26.

B. choleræ.

Culture, "Manila;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.210 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	(a)
1 minute after immersion	45	6,600
1¼ minutes after immersion	48	628
1⅔ minutes after immersion	50	546
2 minutes after immersion	51	0
2¼ minutes after immersion	52	0
2¾ minutes after immersion	53	0
4 minutes after immersion	54	0
5 minutes after immersion	55	0

[a] Innumerable.

EXPERIMENT NO. 27.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(a)
1½ minutes after immersion	45	1,164
2 minutes after immersion	50	32
2½ minutes after immersion	52	0
2¾ minutes after immersion	53	0
3¾ minutes after immersion	54	0
5 minutes after immersion	55	0
6 minutes after immersion	1 minute at 55	0
7 minutes after immersion	2 minutes at 55	0
8 minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 28.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.198 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(a)
1½ minutes after immersion	45	(b)
2 minutes after immersion	50	245
2½ minutes after immersion	52	205
3 minutes after immersion	53	22
3¾ minutes after immersion	54	0
4¾ minutes after immersion	55	0
5¾ minutes after immersion	1 minute at 55	0
6¾ minutes after immersion	2 minutes at 55	0
7¾ minutes after immersion	3 minutes at 55	0

[a] Innumerable.
[b] Innumerable, but less.

EXPERIMENT No. 29.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(a)
1½ minutes after immersion	45	1,680
2 minutes after immersion	50	244
2¼ minutes after immersion	52	47
2¾ minutes after immersion	53	0
3¼ minutes after immersion	54	0
4¾ minutes after immersion	55	0
5¾ minutes after immersion	1 minute at 55	0
6¾ minutes after immersion	2 minutes at 55	0
7¾ minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 30.

B. choleræ.

Culture, "Manila;" twenty-four hours' growth in sterilized milk. Reaction, 0.181 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Number colonies per loop in gelatin.
At start	24	(*a*)
1½ minutes after immersion	45	4,455
2 minutes after immersion	50	1,100
2¾ minutes after immersion	52	90
3 minutes after immersion	53	7
3¾ minutes after immersion	54	0
5¼ minutes after immersion	55	0
6¼ minutes after immersion	1 minute at 55	0
7¼ minutes after immersion	2 minutes at 55	0
8¼ minutes after immersion	3 minutes at 55	0

[a] Innumerable.

EXPERIMENT No. 31.

B. choleræ.

Culture, "Hamburg;" two days' growth in sterilized milk. Reaction, 0.189 per cent acid.

Milk immersed at 25.3° C. in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	25.3	Growth.
1½ minutes after immersion	45	Do.
2 minutes after immersion	50	No growth.
2¼ minutes after immersion	51	Do.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53	Do.
3¼ minutes after immersion	54	Do.
4½ minutes after immersion	55	Do.
5½ minutes after immersion	1 minute at 55	Do.
6½ minutes after immersion	2 minutes at 55	Do.
7½ minutes after immersion	3 minutes at 55	Do.

Experiment No. 32.

B. choleræ.

Culture, "Manila:" forty-eight hours' growth in sterilized milk. Reaction, 0.180 per cent acid.

Milk at 26.3° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bouillon.
At start	26.3	Growth.
1⅓ minutes after immersion	45	Do.
1¾ minutes after immersion	50	Do.
2¼ minutes after immersion	51	No growth.
2½ minutes after immersion	52	Do.
2¾ minutes after immersion	53	Do.
3¼ minutes after immersion	54	Do.
4¼ minutes after immersion	55	Do.
5¼ minutes after immersion	1 minute at 55	Do.
6¼ minutes after immersion	2 minutes at 55	Do.
7¼ minutes after immersion	3 minutes at 55	Do.

Experiment No. 33.

B. choleræ.

Culture, "Hamburg;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.216 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.
At start	31	Growth.
1 minute after immersion	45	Do.
1½ minutes after immersion	48	No growth.
1⅔ minutes after immersion	50	Growth.
1¾ minutes after immersion	51	No growth.
2 minutes after immersion	52	Growth.
2⅓ minutes after immersion	53	No growth.
3 minutes after immersion	54	Do.
4⅔ minutes after immersion	55	Do.

Experiment No. 34.

B. choleræ.

Culture, "Manila;" seventy-two hours' growth in sterilized milk. Reaction of milk, 0.210 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c. c. of milk culture into bouillon.
At start	31	Growth.
1 minute after immersion	45	Do.
1½ minutes after immersion	48	Do.
1⅔ minutes after immersion	50	Do.
1¾ minutes after immersion	51	Do.
2 minutes after immersion	52	Do.
2⅓ minutes after immersion	53	Do.
3 minutes after immersion	54	(Lost.)
4⅔ minutes after immersion	55	No growth.

EXPERIMENT NO. 35.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in sterilized milk. Reaction, 0.140 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1⅔ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2¼ minutes after immersion	55	Do.
2¾ minutes after immersion	57	No growth.
3½ minutes after immersion	59	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT NO. 36.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in sterilized milk. Reaction, 0.09 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1⅔ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2¼ minutes after immersion	55	No growth.
2¾ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.

EXPERIMENT NO. 37.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in sterilized milk. Reaction, 0.189 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon
At start	26	Growth.
1½ minutes after immersion	52	Do.
2 minutes after immersion	55	Do.
5 minutes after immersion	60	No growth.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

EXPERIMENT No. 38.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in sterilized milk. Reaction, 0.16 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
1½ minutes after immersion	52	Do.
2 minutes after immersion	55	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	No growth.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

EXPERIMENT No. 39.

B. choleræ.

Culture, "Hamburg;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.243 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C).	1 c. c. milk culture in bouillon.
At start	21	Growth.
1¼ minutes after immersion	50	Do.
1½ minutes after immersion	53	Do.
1¾ minutes after immersion	55	Do.
2¾ minutes after immersion	57	No growth.
3 minutes after immersion	59	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.
7½ minutes after immersion	3 minutes at 60	Do.

EXPERIMENT No. 40.

B. choleræ.

Culture, "Manila;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.234 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	53	Do.
2¼ minutes after immersion	55	No growth.
2¾ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.

Conclusion.—The cholera vibrio is similar to the diphtheria bacillus so far as its thermal death point is concerned. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

BACILLUS DYSENTERIÆ.

The only reference to the thermal death point of the *B. dysenteriæ* I have been able to find is the work done under Kolle's direction by Friedel, Kutscher, and Meinicke,[a] who found that the dysentery bacillus withstands a temperature of 60° for eight minutes, but is killed at 65° for two minutes. In these experiments it required five minutes to heat the milk from 15° C. to the temperature noted.

I used eight different cultures of the dysentery bacillus, representing both the Shiga and the Flexner types. My results are given in the following tables:

EXPERIMENT NO. 41.

B. Dysenteriæ.

Three days' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in bullion.	Number of colonies per loop in agar.
"Duval;" reaction, 0.126 per cent acid:			
At start	28	Growth	([b])
1¾ minutes after immersion	55	do	1,800
2⅓ minutes after immersion	58	do	28
3¾ minutes after immersion	60	No growth	0
4¾ minutes after immersion	([c])	do	0
"Sils;" reaction, 0.162 per cent acid:			
At start	29	Growth	([b])
1⅔ minutes after immersion	55	do	1,110
2¼ minutes after immersion	58	do.[d]	56
3 minutes after immersion	60	No growth	0
4 minutes after immersion	([c])	do	0
"Kruse;" reaction, 0.187 per cent acid:			
At start	28	Growth	([b])
1⅔ minutes after immersion	55	do	126
2⅓ minutes after immersion	58	do.[d]	0
3⅔ minutes after immersion	60	No growth	0
4⅔ minutes after immersion	([c])	do	0
"Mt. Desert;" reaction, 0.198 per cent acid:			
At start	28	Growth	([b])
1¾ minutes after immersion	55	do	57
2½ minutes after immersion	58	No growth	14
4¼ minutes after immersion	60	do	0
5¼ minutes after immersion	([c])	do	0
"Salant;" reaction, 0.189 per cent acid:			
At start	29	Growth	([b])
1½ minutes after immersion	55	do	([b])
2¼ minutes after immersion	58	do	154
3⅔ minutes after immersion	60	No growth	0
4⅔ minutes after immersion	([c])	do	0

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[a] Friedel, Kutscher, and Meinicke: Die Widerstandsfähigkeit der Erreger der wichtigsten Darmkrankheiten gegen Erwärmen auf verschiedene Temperaturen in Milch. Klin. Jahrb., vol. 3, 1904–5, p. 34.

[b] Innumerable.

[c] One minute at 60°.

[d] Delayed.

EXPERIMENT NO. 42.

B. dysenteriæ.

Culture, "Salent:" forty-eight hours' growth in sterilized milk. Reaction, 0.18 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1¾ minutes after immersion	50	Do.
2½ minutes after immersion	55	Do.
3 minutes after immersion	57	Do.
3½ minutes after immersion	58	Do.
4½ minutes after immersion	59	Do.
5¾ minutes after immersion	59	Do.
6¾ minutes after immersion	60	Do.
7¾ minutes after immersion	1 minute at 60	Do.

EXPERIMENT NO. 43.

B. dysenteriæ.

Culture, "Sils:" forty-eight hours' growth in sterilized milk. Reaction, 0.176 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	24	Growth.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3 minutes after immersion	58	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.

EXPERIMENT NO. 44.

B. dysenteriæ.

Culture, "Salant:" forty-eight hours' growth in sterilized milk. Reaction. 0.135 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2¼ minutes after immersion	58	Do.
3¾ minutes after immersion	60	Do.
4¾ minutes after immersion	1 minute at 60	Do.
5¾ minutes after immersion	2 minutes at 60	No growth.
6¾ minutes after immersion	3 minutes at 60	Do.
7¾ minutes after immersion	4 minutes at 60	Do.
8¾ minutes after immersion	5 minutes at 60	Do.

EXPERIMENT NO. 45.

B. dysenteriæ.

Culture, "Salant;" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
2 minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	No growth.
13 minutes after immersion	8 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.
20 minutes after immersion	15 minutes at 60	Do.

Experiment No. 46.

B. dysenteriæ.

Culture "Duval;" forty-eight hours' growth in sterilized milk. Reaction, 0.108 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2½ minutes after immersion	58	Do.
4½ minutes after immersion	60	Do.
5½ minutes after immersion	1 minute at 60	Do.
6½ minutes after immersion	2 minutes at 60	Do.
7½ minutes after immersion	3 minutes at 60	No growth.
9½ minutes after immersion	5 minutes at 60	Do.
14½ minutes after immersion	10 minutes at 60	Do.

Experiment No. 47.

B. dysenteriæ.

Culture, "Sils;" forty-eight hours' growth in sterilized milk. Reaction, 0.165 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (°C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
2 minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	No growth.
7 minutes after immersion	2 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	Do.
13 minutes after immersion	8 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.
20 minutes after immersion	15 minutes at 60	Do.

EXPERIMENT NO. 48.

B. dysenteriæ.

Culture, "Kruse:" forty-eight hours' growth in sterilized milk. Reaction, 0.144 per cent acid.

The milk at 24° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. (° C.).	⅓ c. c. milk culture in bouillon.
At start	24	Growth.
1½ minutes after immersion	50	Do.
2¼ minutes after immersion	55	Do.
3 minutes after immersion	58	Do.
3¾ minutes after immersion	60	Do.
4¾ minutes after immersion	60.5	No growth.
5¾ minutes after immersion	61	Do.
6¾ minutes after immersion	61.5	Do.
7¾ minutes after immersion	61.5	Do.
8¾ minutes after immersion	61	Do.

EXPERIMENT NO. 49.

B. dysenteriæ.

Culture, "Witaus:" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	25	Growth.
1¼ minutes after immersion	50	Do.
2 minutes aftes immersion	55	Do.
3 minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	No growth.

EXPERIMENT NO. 50.

B. dysenteriæ.

Culture. "Wilson;" forty-eight hours' growth in sterilized milk. Reaction, 0.144 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	25	Growth.
1⅓ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2¾ minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	No growth.

EXPERIMENT NO. 51.

B. dysenteriæ.

Culture. "Mt. Desert;" forty-eight hours' growth in sterilized milk. Reaction, 0.252 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
1¼ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2½ minutes after immersion	58	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	No growth.
15 minutes after immersion	10 minutes at 60	Do.

Experiment No. 52.

B. dysenteriæ.

Culture, "Harris;" forty-eight hours' growth in sterilized milk. Reaction, 0.234 per cent acid.

The milk at 26° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	½ c. c. milk culture in bouillon.
At start	26	Growth.
1½ minutes after immersion	50	Do.
2 minutes after immersion	55	Do.
2¾ minutes after immersion	58	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	Do.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	No growth.
14¼ minutes after immersion	10 minutes at 60	Do.

Experiment No. 53.

B. dysenteriæ.

Culture, "Sils;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.288 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	Do.
10 minutes after immersion	5 minutes at 60	No growth.
15 minutes after immersion	10 minutes at 60	Do.

EXPERIMENT NO. 54.

B. dysenteriæ.

Culture, "Shiga;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.27 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3¼ minutes after immersion	59	Do.
4¼ minutes after immersion	60	Do.
5¼ minutes after immersion	1 minute at 60	Do.
6¼ minutes after immersion	2 minutes at 60	Do.
7¼ minutes after immersion	3 minutes at 60	Do.
9¼ minutes after immersion	5 minutes at 60	Do.
14¼ minutes after immersion	10 minutes at 60	No growth.

EXPERIMENT NO. 55.

B. dysenteriæ.

Culture, "Duval;" forty-eight hours' growth in whole milk that had been sterilized by the fractional method three days. Reaction, 0.306 per cent acid.

The milk at 21° C. immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature. (° C.).	1 c. c. milk culture in bouillon.
At start	21	Growth.
2 minutes after immersion	55	Do.
2½ minutes after immersion	57	Do.
3½ minutes after immersion	59	Do.
5 minutes after immersion	60	Do.
6 minutes after immersion	1 minute at 60	Do.
7 minutes after immersion	2 minutes at 60	Do.
8 minutes after immersion	3 minutes at 60	No growth.
10 minutes after immersion	5 minutes at 60	Do.
15 minutes after immersion	10 minutes at 60	Do.

Conclusion—The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at 60° for five minutes. All are killed at 60° C. for ten minutes. However, the great majority of these micro-organisms are killed by the time the milk reaches 60° C.

MICROCOCCUS MELITENSIS.

Maj. W. H. Horrocks and Capt. J. C. Kennedy, of the British Army Medical Corps, in their article upon goats as a means of propagating Mediterranean fever,[a] made several experiments to determine whether it is possible to destroy the *M. melitensis* by pasteurization of the infected milk, and concluded that heating the milk to 68° C. for ten minutes destroyed the *M. melitensis* present in infected goats' milk. No record, however, is made of experiments with lower temperatures.

Experiment 1.—Milk was drawn from goats 1, 2, 3, 5, and 6 and thoroughly mixed. This mixed milk was selected for experiment as it contained thick, ropy masses, and it was thought that these might act as protecting envelopes to the specific micrococci, so that if such a milk were sterilized by pasteurization it might safely be concluded that any ordinary normal-looking milk would be equally sterilized by the same operation.

One loopful of the mixed milk was stroked on a series of surface plates to act as a control. The mixed milk was then heated on a water bath to 68° C., and this temperature was maintained for ten minutes. The milk was then rapidly cooled and 10 c. c. of the sterilized milk were spread over 20 litmus-nutrose-agar plates.

Results: After four days incubation at 37° C. the control plates were found densely crowded with colonies of the *M. melitensis;* the plates made from the pasteurized milk remained free from any signs of the specific *Micrococcus melitensis*, even though the incubation was continued for fourteen days.

Experiment 2.—This served as a control of experiment 1, the same procedure being followed. The *Micrococcus melitensis* appeared in the control plates, but the plates made with the pasteurized milk again remained perfectly sterile.

In my experiments I used two strains of the *M. melitensis*. The results follow:

Experiment No. 56.

M. melitensis.

Culture, seven days' growth in bouillon, mixed with milk that had been inoculated the previous day with same culture. Reaction of milk, 0.081 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	Loop of milk culture in agar.
At start	31	Innumerable.
1¼ minutes after immersion	45	Do.
1½ minutes after immersion	48	Do.
1¾ minutes after immersion	50	Do.
2 minutes after immersion	51	Do.
2¼ minutes after immersion	52	Do.
2½ minutes after immersion	53	Do.
3 minutes after immersion	54	Do.
4 minutes after immersion	55	Do.

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[a] Horrocks, W. H., and Kennedy, J. C.: VI. Goats as a means of propagation of Mediterranean fever. Reports of the commission * * * for the investigation of Mediterranean fever * * * Part 4. London, Harrison & Sons, Feb., 1906. 8° Paper. 187 p.

EXPERIMENT NO. 57.

M. melitensis.

Culture, seven days' growth in bouillon, mixed with milk that had been inoculated the previous day with same culture. Reaction of milk, 0.081 per cent acid.

The milk at 31° C. immersed in constant-temperature water bath at 55° C.

Milk culture.	Temperature (° C.).	About ⅓ c.c. of milk culture into bouillon.	Loop of milk culture in bouillon.
At start	31	Growth	Growth.
1 minute after immersion	45	do	Do.
1½ minutes after immersion	48	do	Do.
1⅔ minutes after immersion	50	do	Do.
2 minutes after immersion	51	do	Do.
2¼ minutes after immersion	52	do	Do.
2½ minutes after immersion	53	do	Do.
3 minutes after immersion	54	do	Do.
4¼ minutes after immersion	55	do	Do.

EXPERIMENT NO. 58.

M. melitensis.

Five days' growth in sterilized milk. Milk immersed in constant-temperature water bath at 60° C.

Milk culture.	Temperature (° C.).	Number of colonies per loop in agar.
"Stock" culture; reaction, 0.108 per cent acid:		
At start	30	Innumerable.
½ minute after immersion	45	Do.
1 minute after immersion	50	Do.
1¼ minutes after immersion	55	Do.
1¾ minutes after immersion	58	53.
2 minutes after immersion	60	0
3 minutes after immersion	1 minute at 60	0
"U. S. N." culture; reaction, 0.072 per cent acid:		
At start	33	Innumerable.
½ minute after immersion	45	Do.
1 minute after immersion	50	Do.
1⅓ minutes after immersion	55	Do.
2 minutes after immersion	58	Do.
2¾ minutes after immersion	60	0
3¾ minutes after immersion	1 minute at 60	0

NOTE.—Agar plates incubated five days at 37° C.

Conclusion.—So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. The *M. melitensis* is not destroyed at 55° for a short time; the great majority die at 58°; at 60° all are killed.

GENERAL SUMMARY AND CONCLUSIONS.

It is difficult to determine precisely at what moment or at what temperature a micro-organism dies. The fact that a micro-organism will not grow upon artificial media is not always a sure sign that it is dead; but with the exception of the tubercle bacillus it is safe to assume that ordinarily bacteria that fail to vegetate upon suitable media under favorable conditions have at least lost their virulence and power to infect, especially when ingested by the mouth.

We know, however, that the vegetability of micro-organisms *in vitro* does not always correspond to their ability to grow in the animal host. This is especially true of the tubercle bacillus. On account of the reluctance with which this bacillus grows upon culture media, it is necessary to resort to animal inoculations in order to determine its thermal death point. It is proper to assume that the tubercle bacilli in milk which are so attenuated as to be unable to cause tuberculosis when injected into the peritoneal cavity of a young guinea pig would be harmless when ingested by man.

In these experiments upon the guinea pig it is important to differentiate the lesions produced by dead tubercle bacilli, which closely simulate those caused by the live tubercle bacilli. In doubtful cases it is necessary to inoculate the products of the lesions into another animal to determine the presence or absence of living micro-organisms.

The cultural experiments upon thermal death points are surrounded by many sources of error and numerous pitfalls, all of which must be avoided.

My experiments were designed to imitate the conditions of practical pasteurization. The micro-organisms were heated in open test tubes and the temperature and other factors accurately controlled. Scum formation was disregarded, as it was my intention to reach results that might be applied with confidence in practical pasteurization on a large scale against natural difficulties.

My results of nine series of tests upon guinea pigs with five cultures plainly show that in milk the tubercle bacillus loses its virulence and infective power when heated at 60° for twenty minutes; in other words, it may be considered dead. When heated to 65° C. a much shorter time is necessary. These results correspond in the main with those previously recorded by Yersin, Bonhoff, Schroeder, Th. Smith, Russell and Hastings, and Hesse.

It should be remembered that the milk in these tests was very heavily infected with virulent cultures, indicated by the prompt death of the control animals. Milk practically never contains such an enormous amount of infection under natural conditions. It is justifiable, therefore, to assume that if 60° C. for twenty minutes is sufficient to destroy the infectiousness of such milk when injected into the peritoneal cavity of a guinea pig, any ordinary market milk after such treatment would be safe for human use by the mouth so far as tubercle bacilli are concerned.

The lesions produced by a large mass of dead tubercle bacilli may be distinguished by their extent rather than by their character. In doubtful cases secondary inoculation is the only trustworthy method of determining whether the bacilli are alive or dead. The tuberculin test does not differentiate between the live and dead tubercles. Three guinea pigs out of eight having lesions produced by dead tubercle bacilli (killed at 100° C.) died as the result of the subcutaneous inoculation of 2 c. c. tuberculin (O. T.).

The evidence is plain that milk heated to 60° C. and maintained at that temperature for two minutes will kill the typhoid bacillus. The great majority of these organisms are killed by the time the temperature reaches 59° C., and few survive to 60° C.

The diphtheria bacillus succumbs at comparatively low temperatures. Oftentimes it fails to grow after heating to 55° C. Some occasionally survive until the milk reaches 60° C.

The cholera vibrio is similar to the diphtheria bacillus so far as its thermal death point is concerned. It is usually destroyed when the milk reaches 55° C.; only once did it survive to 60° C. under the conditions of the experiments.

The dysentery bacillus is somewhat more resistant to heat than the typhoid bacillus. It sometimes withstands heating at 60° C. for five minutes. All are killed at 60° C. for ten minutes. However, the great majority of these micro-organisms are killed by the time the milk reaches 60° C.

So far as can be judged from the meager evidence at hand, 60° C. for twenty minutes is more than sufficient to destroy the infective principle of Malta fever in milk. The *M. melitensis* is not destroyed at 55° C. for a short time; the great majority of these organisms die at 58°, and at 60° all are killed.

Milk heated to 60° C. and maintained at that temperature for twenty minutes may, therefore, be considered safe so far as conveying infection with the micro-organisms tested is concerned.

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The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued.

No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

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No. 5.—An investigation of a pathogienic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

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No. 40.—Miscellaneous Zoological Papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

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TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.

HYGIENIC LABORATORY.—BULLETIN No. 43.
MARCH, 1908.

THE STANDARDIZATION OF TETANUS ANTITOXIN

(AN AMERICAN UNIT ESTABLISHED UNDER
AUTHORITY OF THE ACT OF JULY 1, 1902)

BY

M. J. ROSENAU
and
JOHN F. ANDERSON.

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1908.

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WALTER WYMAN, *Surgeon-General,*
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THE STANDARDIZATION OF TETANUS ANTITOXIN[a]

[*An American unit established under authority of the act of July 1, 1902.*]

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INTRODUCTION.

There are now four methods of measuring the strength of tetanus antitoxin: (1) The German method described by Behring, (2) the French method of Roux, (3) the Italian method after Tizzoni, and (4) the American method described in this bulletin.

The European standards are admitted to be unsatisfactory and, for the most part, not accurate; further, they are complicated and difficult to carry out. The American method adopted officially under the law of July 1, 1902, for this country is the result of several years, work upon this subject in the Hygienic Laboratory and it is believed will commend itself for its simplicity, directness, and accuracy.

The standard toxines and antitoxins are preserved under special precautions to prevent deterioration and are tested against each other reciprocally, so that the least alteration in either may be detected.

While the unit is based upon the neutralizing value of an arbitrary quantity of antitoxic serum, the *antitoxin* is not issued to other laboratories for the purposes of test, as is the case with diphtheria. A stable precipitated tetanus *toxine*, the test dose of which has been carefully determined, is given out. All the tetanus antitoxic serums for use in man upon the American market are now measured against this same standard toxine and have therefore precisely comparable strengths.

The need of uniformity in standardizing this valuable prophylactic serum is evident from the table on page 11, from which it will be seen that before the establishment of an American standard the tetanus serums upon the market varied extravagantly in the unit strength claimed, and were, for the most part, comparatively weak in true antitoxic potency. Since the promulgation of this standard unit the serums on the market have decidedly greater antitoxic value. The feebleness of the foreign serums compared with those of American manufacture is also evident from the table.

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[a] Manuscript submitted for publication March 4, 1908.

The object of this bulletin is to describe in detail the methods used in preparing and using this standard. A discussion of the theoretical considerations is introduced for a better understanding of the principles involved.

AMERICAN UNIT, AND METHODS OF STANDARDIZATION.

Definitions.—"The immunity unit for measuring the strength of tetanus antitoxin shall be ten times the least quantity of antitetanic serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine-Hospital Service."

The unit was thus officially defined October 25, 1907, in Treasury Department Circular No. 61. This circular amended the regulations promulgated in accordance with the act approved July 1, 1902, entitled "An act to regulate the sale of viruses, serums, toxins, and analogous products in the District of Columbia, to regulate interstate traffic in said articles, and for other purposes."

Based largely upon the work on the standardization of tetanus antitoxin done in the Hygienic Laboratory, a special committee of the Society of American Bacteriologists, which met in New York December 27 and 28, 1906, made the following report, which was unanimously adopted:

> That tetanus antitoxin be standardized by the tetanus toxin furnished by the Public Health and Marine-Hospital Service. The unit is ten times the least amount of serum necessary to save the life of a 350-gram guinea pig for ninety-six hours against the official test dose of the standard toxin. The test dose is 100 minimal lethal doses of a precipitated toxin preserved under special conditions at the Hygienic Laboratory of the Public Health and Marine-Hospital Service. It was decided that the minimal immunizing dose for a case of possible infection through a wound should be 1,500 of such units. It was decided that after April 1 the new unit should be adopted by all producers of tetanus antitoxin.
>
> J. J. Kinyoun, *Chairman.*
> Theobald Smith.
> Herbert D. Pease.
> E. M. Houghton.
> Joseph McFarland.
> M. J. Rosenau.
> William H. Park, *Secretary.*

The American unit for measuring the strength of tetanus antitoxin may be defined as the neutralizing power possessed by an arbitrary quantity of antitetanic serum preserved under special conditions to prevent deterioration in the Hygienic Laboratory of the Public Health and Marine-Hospital Service. This arbitrary quantity now contains ten times the amount of tetanus antitoxin necessary to neutralize somewhat less than 100 minimal lethal doses of a standard toxine for a 350-gram guinea pig—that is, one-tenth of a unit mixed with 100 minimal lethal doses of the standard toxine contains just enough free poison in the mixture to kill the guinea pig in four days after subcutaneous injection.

The standardization of tetanus antitoxin does not differ radically from the standardization of diphtheria antitoxin. The toxines and antitoxins are measured against each other reciprocally, so that change or deterioration of either the standard toxine or the standard antitoxin may readily be determined. Duplicate toxines and antitoxins made from time to time will act as checks against deterioration of either the standard toxines or antitoxins.

The value of an unknown serum is measured indirectly through the toxine, using the L+dose as the test dose. The L+dose is the smallest quantity of tetanus toxine that will neutralize one-tenth of an immunity unit, plus a quantity of toxine sufficient to kill the animal in just four days.

The *toxine* and not the *antitoxin* is given out to licensed manufacturers and others interested for the purposes of standardizing their serums. The L+ or test dose of the particular toxine (A) now dispensed contains just 100 minimal lethal doses for a 350-gram guinea pig. This particular toxine is very stable and has not changed appreciably in two years. As soon as it alters or is exhausted the next toxine that will be issued may contain considerably more or less than 100 minimal lethal doses, but the test dose will contain precisely the same neutralizing power.

The antitoxic serum, for the purposes of this standard, was obtained from a single horse. The serum was reduced to dryness and ground to an impalpable powder. The powder so obtained is preserved in many vacuum tubes under the influence of pentaphosphoric acid. These tubes are kept in absolute darkness at a constant temperature of 5° C. Every two months, or oftener, one of these tubes is opened, dissolved in a solution of glycerin 66 parts and isotonic salt solution 34 parts, and tested.

While the tetanus antitoxin preserved in dry powdered form under the conditions above named is stable, duplicates of the antitoxins made from time to time so as to guard against loss or change will insure the permanence of the standard which has now been established.

METHODS.

In order to obtain reliable and comparable results, it is necessary to take into account all the factors concerned—the composition of the poisons, their concentration, the diluting fluid, length of time the mixtures are allowed to stand, the site of inoculation, etc. All the known factors have been considered in our method of standardizing tetanus antitoxin, so that it is our belief that we now have as accurate and satisfactory a standard for this antitoxin as is the case with diphtheria.

Careful regard must be had for the following points in order to obtain uniform results:

The diluting fluid.—Salt solution containing 0.85 per cent chemically pure sodium chlorid sterilized by boiling is used for diluting both the toxine and the antitoxin.

Time and temperature.—The mixtures of toxine and antitoxin are kept one hour at room temperature in diffused light before injection into the guinea pig.

Amount.—A total of 4 c. c. of the toxine-antitoxin mixture is injected into the guinea pig. If the toxine-antitoxin mixture does not equal 4 c. c., salt solution equal in amount to the difference is used to wash out the syringe, so that the total amount injected and the pressure effects are always equal.

Concentration of the toxine.—One-tenth of a gram of the dried tetanus toxine (A) is dissolved in 166.66 c. c. salt solution. One c. c. of this dilution equals the test dose (L + dose).

Concentration of the antitoxin.—The dilution of the antitoxic serum should follow the tables on page 50.

Guinea pigs.—The guinea pigs should weigh 350 grams. Animals weighing from 300 to 400 grams are allowable in preliminary rough testing.

Site of injection.—The injection is always given subcutaneously into the tissues of the abdomen about the level of the umbilicus.[a]

Time of death.—The number of immunity units contained in the serum is determined from the amount given the animals that are living ninety-six hours after the inoculation of the mixtures.

An example of a test.—Carefully tare a weighing bottle, then add approximately 20 to 50 mg. of the dried poison. Again carefully weigh. Dissolve the toxine in the weighing bottle with salt solution (0.85) in the proportion of 0.1 gram of the dried poison to 166.66 c. c. of the salt solution. This proportion is used for the reason that each cubic centimeter of this solution will represent 0.000,6 gm. of the original dried poison (=100 MLD's). This proportion is taken because it is very convenient in measuring out the test dose, which represents 1 c. c. of the solution. Thus:

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[a] It is very important to inject the animals in the same place on account of the relation to the motor nerve endings and the distance to travel to the central nervous system.

44.5692 gm., bottle + toxine.
44.5300 gm., bottle.

.0392 gm., toxine.
0.1 gm. : 166.66 c. c. :: 0.0392 : x.
x = 65.33 c. c.

In other words, if the quantity of toxine placed in the weighing bottle should weigh, as in this instance, just 0.0392 gm., carefully deliver from an accurately graduated burette just 65.33 c. c. salt solution into the weighing bottle; and, as before stated, each cubic centimeter of this solution will be the L + or test dose.

Now dissolve the serum of unknown value in accordance with the table of dilutions (page 50) and mix aliquot parts of the serum with the test dose of toxine, as follows:

TABLE No. 1.

No. of guinea pig.	Weight of guinea pig (grams).	Subcutaneous injection of a mixture of—		Time of death.
		Toxine (test dose).	Antitoxin.	
		Gram.	*c. c.*	
1	360	0.0006	0.001	2 days, 4 hours.
2	350	.0006	.0015	4 days, 1 hour.
3	350	.0006	.002	Symptoms.
4	360	.0006	.0025	Slight symptoms.
5	350	.0006	.003	No symptoms.

According to this series the guinea pig which received 0.0015 c. c. of the serum died in four days and one hour. Therefore 0.0015 c. c. of the serum contains one-tenth of an immunity unit, as the unit has been defined as ten times the least amount of antitetanic serum necessary to save the life of a 350-gram guinea pig 96 hours against the official test dose. This serum would, therefore, contain just 66 units per cubic centimeter.

On account of the importance of the L+ or test dose the following table is given showing the results of these tests with Toxine A.

TABLE No. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908.*

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
306	290	Jan. 17, 1907	0.0006	1 unit	4	0
326	300	Jan. 24, 1907	.0006	1 unit	3	3
334	315	do	.0006	1 unit	3	7
387	350	Feb. 20, 1907	.0006	1 unit	4	19
388	350	do	.0006	1 unit	4	8
389	350	do	.0006	1 unit	4	16

Table No. 2.—*Showing the results of the test (L+) dose of tetanus toxine A from January 17, 1907, to April 7, 1908*—Continued.

Guinea pig No.	Weight in grams.	Date.	Amount of toxine A.	Amount of antitoxin.	Time of death.	
			Gram.		*Days.*	*Hours.*
400	330	Feb. 25, 1907	0.0006	1 unit	4	21
401	315	do	.0006	1 unit	5	7
407	335	do	.0006	1 unit	4	16
408	315	do	.0006	1 unit	4	9
415	300	Mar. 5, 1907	.0006	1 unit	3	0
416	325	do	.0006	1 unit	3	10
422	300	do	.0006	1 unit	3	9
423	325	do	.0006	1 unit	3	10
430	290	Mar. 22, 1907	.0006	1 unit	4	11
431	290	do	.0006	1 unit	4	14
454	300	Mar. 28, 1907	.0006	1 unit	4	1
455	300	do	.0006	1 unit	3	18
509	300	Apr. 13, 1907	.0006	1 unit	3	17
510	295	do	.0006	1 unit	3	17
524	280	May 3, 1907	.0006	1 unit	3	18
525	290	do	.0006	1 unit	3	11
538	300	May 17, 1907	.0006	1 unit	3	20
539	320	do	.0006	1 unit	3	7
556	330	May 24, 1907	.0006	1 unit	2	20
557	350	do	.0006	1 unit	3	10
615	375	July 28, 1907	.0006	1 unit	2	22
616	380	do	.0006	1 unit	3	10
625	350	July 31, 1907	.0006	1 unit	3	13
626	350	do	.0006	1 unit	4	12
639	350	Sept. 6, 1907	.0006	1 unit	3	4
640	350	do	.0006	1 unit	3	2
654	325	Sept. 24, 1907	.0006	1 unit	3	17
655	370	do	.0006	1 unit	6	19
679	325	Nov. 11, 1907	.0006	1 unit	3	19
680	350	do	.0006	1 unit	4	0
683	350	Dec. 7, 1907	.0006	1 unit	3	15
684	360	do	.0006	1 unit	3	13
702	370	Dec. 19, 1907	.0006	1 unit	3	23
757	350	Apr. 7, 1908	.0006	1 unit	4	1
758	350	do	.0006	1 unit	3	18
759	350	do	.0006	1 unit	4	0

NECESSITY FOR THIS STANDARD.

We now know that when the symptoms of tetanus have developed the toxine has combined with the motor nerve cells and that the union between the cell and the poison is too strong for the antitoxin to break up. However, if the antitoxin is given in time the toxine is neutralized and rendered harmless. Tetanus antitoxin is therefore an exceedingly valuable prophylactic and for this purpose is coming into more general use both in human and veterinary practice.

Whether used as a prophylactic or curative agent it is important to use a potent serum. The law of July 1, 1902,[a] not only insures serums of useful potency, but also establishes uniformity among the producers of tetanus antitoxin in America. The necessity of establishing uniformity for this product in American markets is evident from the following table:

TABLE NO. 3.—*Showing the differences in the methods of testing tetanus antitoxin before the adoption of the American unit.*

Name.	Labeled.	Contents (cubic centimeters).	Units claimed according to special methods of standardization, per cubic centimeter.	Units according to the American standard, per cubic centimeter.
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).	One immunizing dose (No. 2929F).		0. 5	434
New York City department of health (submitted by Dr. W. H. Park for comparison).			700	166
H. K. Mulford Co. (bought on open market).	1,000,000 immunizing units (No. 2121).	10	100,000	77
Farbenfabrik vorm. Meister Lucius & Bruning, Hoechst, a/M (submitted for comparative tests).	Tetanus Antitoxin, 5 fach. Normal, Prüfungs dose = 1/500.			333
H. K. Mulford Co., No. 9971 (submitted for tests).	60,000,000 units	10	6,000,000	90
Parke, Davis & Co			60,000	700
New York department of health, Albany (submitted by Dr. H. D. Pease for comparison).			.75	769
Serum Antitetanique, Pasteur Institute, 10 c. c. fluid.	Unit value not stated.	10	Not stated.	40
Serum Antitetanique, Pasteur Institute	Unit value not stated.	10	Not stated.	40
Tizzoni, Antitossina del Tetano, No. 2912, Dry.	Unit value not stated.		Not stated.	[b] 83. 3
Pasteur Institute, Serum antitetanique, II-7.	Unit value not stated.	10	Not stated.	66
Institute Bacteriologique de Lyon et du Sud-Est.	Unit value not stated.	10	Not stated.	(c)

[a] Dissolved in 26. c. c.
[b] This equals 833 units per gram of the dry serum.
[c] Less than 50.

Tetanus antitoxin is made by the following laboratories in the United States:

Parke, Davis & Co., Detroit, Mich. (license No. 1); H. K. Mulford Company, Philadelphia, Pa. (license No. 2); Lederle Antitoxin

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[a] An act regulating the sale of viruses, serums, toxins, and analogous products, etc. Approved July 1, 1902.

Laboratories, New York, N. Y. (license No. 17); New York State department of health, Albany, N. Y.; and the department of health of the city of New York, N. Y.

HISTORICAL REVIEW.

Compared with the major plagues of man lockjaw has always been a rare disease. It is on account of the peculiar and characteristic spasms that it early attracted attention. In the writings of Hippocrates cases of tetanus are described, diagnosed, and prognosis given. From the learned Aretaeus of Kappadoza we have a description of tetanus that holds until modern times.[a] Aretaeus described an opisthotonos, an emphrothotonos, and a tetanus, depending upon whether the muscles of the back, the abdomen or the general muscular system were affected. To this classification pleurothotonos was later added when the muscles of one side were especially affected.

Throughout the Middle Ages our knowledge of tetanus remained at a standstill. The symptoms of the disease so plainly indicated that the lesions are localized in the nervous system that, with the introduction of studies in pathologic anatomy, the brain, spinal cord, and nerves were studied to determine the seat and nature of the illness. It was not, however, until about the end of "the sixties" in the last century that experimental studies in wound infections began to throw light upon the chaos of theories. A review of the historical development of these theories is exceedingly interesting, for they mirror the prevailing thought upon the nature of disease, as it developed from that of evil spirits, through the "humoral" theory, the realm of miasm and noxious effluvia, to the germ theory.

It was long known that tetanus was a complication of wounds. Micheles, 1797, thought it was due to irritation of the peripheral nerves from foul secretions in certain wounds ("wound insults"). Tetanus could not escape the rheumatism theory, which has been such an alluring catch-all for symptoms and diseases difficult of explanation. In cases of wound tetanus in which no plausible explanation seemed possible it was called rheumatic wound tetanus. "Taking cold" was assigned its usual rôle here as elsewhere. When no assignable cause seemed at hand the disease was given the learned title "idiopathic tetanus."

About 1860 Heiberg and Rose, and Billroth and Spencer Wells[b] attached tetanus to the list of zymotic diseases and believed it due to a miasm, the spasms being caused by a poison in the blood similar to strychnine.

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[a] A part of the historical data are taken from Von Lingelsheim's article on Tetanus in Kolle & Wassermann's Handbuch der pathogenen Mikroorganismen, vol. 2, p. 566–600.

[b] Billroth and Spencer Wells. Wiener med. Presse, 1869, p. 36.

E. Rose[a] 1870, described a particular form of tetanus following wounds in the area of the twelfth cerebral nerve which he called "head tetanus" or "tetanus hydrophobicus."

An important milestone in the history of tetanus was passed when Strümpell,[b] 1876, declared tetanus to be an infection. The first experiments to prove this view failed.

Arloing and Tripier[c] did not succeed in transferring the disease to dogs and rabbits by carrying over the blood and the pus from tetanus wounds.

Billroth[d] and Schultz[e] also obtained negative results with dogs.

Carle and Rattone,[f] 1884, were the first to obtain positive results in demonstrating the transmissibility of the disease. They injected a number of rabbits with the washings from the neighborhood of an acne pustule from which the tetanus originated. The rabbits were injected either into the sciatic nerve or into the muscles of the back. After an incubation period of two to three days 11 of the 12 rabbits showed typical tetanic spasms. They also succeeded in carrying the infection from animal to animal.

By injecting earth taken from different places into 140 mice, rabbits, guinea pigs, etc., in studying the microorganisms of soil (ground tetanus), Nicolaier,[g] 1884, produced symptoms of tetanus in 69 of them and always found a slender rod in the pus. Therefore he concluded that there exists a bacillus that causes tetanus in deep wounds of mice, rabbits, and guinea pigs. In cultures Nicolaier was unable to separate this bacillus from the other microorganisms with which it was associated, but inoculation experiments with mixed cultures produced tetanus.

The next year (1885) Nicolaier,[h] working under Flügge in the Göttingen Hygienic Institute, made noteworthy advances upon the subject. He showed that each injection into mice, guinea pigs, and rabbits caused a disease with tetanic spasms, while dogs were refractory. Nicolaier saw in the pus of the wounds this slender bacillus, which he considered the cause of the infection, but again was unable to obtain it in pure culture, although he carried on a mixed culture upon coagulated calf's blood serum.

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[a] Nicolaier, Arthur: Zur Aetiologie des Kopftetanus (Rose). Virch. Arch., vol. 128, 1892, p. 1–19.

[b] Strümpell: Ueber die Ursachen der Erkrankungen des Nervensystems. Arch. f. klin. Med., vol. 35, p. 14–15.

[c] Arloing and Tripier: Gaz. med. de Paris, 1870, p. 337.

[d] Billroth: Allgemeine chirurgische Pathologie und Therapie. Berlin, 1882, p. 504.

[e] Schultz: Ueber einer Kumulation von Tetanusfällen im Stadtkrankenhause zu Rostock. Rostock, 1876, p. 13.

[f] Carle and Rattone: Studio sull' enziologia del tetano (communicazione preventiva). Giorn. dell' R. Accad. di med. di Torino, Marzo, 1884.

[g] Nicolaier, Arthur: Ueber infectiosen Tetanus. Deut. med. Woch., vol. 10, 1884, p. 842–844.

[h] Nicolaier, Arthur: Beiträge zur Aetiologie des Wundstarrkrampfes. Inaug. disserta., Göttingen, 1885.

Soon afterwards Rosenbach,[a] 1886, found a similar bacillus with a round terminal spore in a case complicating frost gangrene in man and transferred the infection to animals in the pus.

In 1889, with the aid of anaerobic technic, Kitasato[b] for the first time grew the bacillus in pure culture and by successful inoculation experiments finally proved that this bacillus was the real cause of tetanus. Kitasato succeeded in isolating the tetanus bacillus, first described by Nicolaier and later by Rosenbach, not alone by understanding anaerobic methods but by taking advantage of its resisting spore. This bacillus appears in the pus of wounds of man and animals having tetanus. It often forms spores in the pus, but if the pus is examined early it may contain spore-free rods. He planted the material containing the bacillus upon agar slants, which were incubated one to two days. The mixed cultures so obtained were heated one hour at 80° C. and then transplanted to agar plates. Kitasato designed for this purpose a special shaped apparatus in which hydrogen was used to replace the air.

Kitasato[c] further showed that the tetanus bacillus is not found in the heart's blood of mice dead of tetanus and therefore concluded that we are dealing with an intoxication and not with an infection.

It was in the following year, 1890, that Behring and Kitasato[d] published their epoch-making work upon the tetanus toxin and tetanus antitoxin, laying the foundation of serum therapy. On account of the great historical and practical importance of this document a transcription of its principal features is given:

> The immunity of rabbits and mice which have been immunized against tetanus depends upon the power of the cell-free serum to render the toxic substances which are produced by the tetanus bacillus harmless.
>
> Experiments show:
>
> 1. The blood of tetanus immune rabbits possesses the property of destroying the tetanus poison.
> 2. This property is destroyed by the extravascular blood and the cell-free serum obtained from it.
> 3. This property is of such a stable nature that it is also effective in the bodies of other animals, so that we are in a position, by means of the transfer of blood serum, to accomplish noteworthy therapeutic results.

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[a] Rosenbach: Zur Aetiologie des Wundstarrkrampfes beim Menschen. Arch. f. klin. Chir., Berl., vol. 34, p. 306–317.

[b] Kitasato, S.: Ueber den Tetanusbacillen. Zeit. f. Hyg., vol. 7, 1889, p. 225.

[c] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, p. 304.

[d] Behring, E., and Kitasato, S.: Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Theiren. Deut. med. Woch., vol. 16, 1890, p. 1113–1114.

4. The property of destroying the tetanus poison is absent in the blood of animals which have not been immunized against tetanus. If the tetanus poison be given to susceptible animals it may be demonstrated in the blood and other body fluids after the death of the animal.

For instance: A rabbit was immunized against tetanus to such a high degree of immunity that it was able to withstand 10 c. c. of a virulent culture containing the tetanus organism, 0.5 c. c. of which was sufficient to kill a normal rabbit. This rabbit was not only able to withstand the infection of living tetanus bacilli, but was also immune to the tetanus poison. Thus it withstood without any symptoms twenty times the quantity of tetanus poison sufficient to kill a normal rabbit.

This rabbit was bled from the carotid, and before the blood clotted 0.2 c. c. was given to a mouse and 0.5 c. c. to another. After twenty-four hours both of these treated mice and two control mice were inoculated with a virulent tetanus bacillus. The amount injected was so great that the control animals showed tetanus in twenty hours and were dead in thirty-six hours. Both treated mice, on the other hand, remained well.

The larger quantity of blood was allowed to stand until the serum separated. Of this serum six mice were injected with 0.2 c. c. each into the peritoneal cavity. Twenty-four hours later they were infected and remained well, while the control mice died in less than forty-eight hours.

It was further found that the serum had a therapeutic value in that the mice were first infected and afterwards protected by injecting the serum into the peritoneal cavity.

They further made experiments to demonstrate that the serum has an enormous power of destroying the poison.

Of a tetanus culture ten days old which was freed from bacilli by filtration, 0.00005 c. c. was sufficient to kill a mouse in four to six days and 0.0001 c. c. was sufficient to kill a mouse with certainty in two days. However, 5 c. c. of the serum from the tetanus immune rabbit was mixed with 1 c. c. of this culture, and the serum was allowed to act twenty-four hours upon the culture containing the tetanus poison. Of this mixture, four mice were given each 0.2 c. c., containing 0.0033 c. c. of the culture, or more than 300 times the dose otherwise fatal for mice. All four of the mice remained well. The control mice, on the other hand, died in thirty-six hours with 0.0001 c. c. of the fluid. The mice which were treated as above described, as well as those which had received the mixture of tetanus poison with serum, so far as can be told, remained well. Later they were injected repeatedly with virulent tetanus bacilli, which they withstood without a trace of symptoms.

This fact is of very particular note, because in the innumerable experiments no mouse, no rabbit, in fact no animal so far tested has shown a natural immunity against tetanus. We therefore may draw the conclusion that the above-mentioned explanation of the conditions of immunity which may be obtained promptly and without difficulty, and which may be accomplished without harm to the animals in the process of immunization, will prove of far-reaching value. It is to be understood that control animals were tested with the blood and serum of nonimmune rabbits. The same may be said to be true of cattle and sheep.

We permit ourselves from our results to venture the conclusion that, after finding a therapeutically potent substance useful for animals, it will also be useful in the treatment of diphtheria and tetanus in man. We only desire to call attention to one thing in conclusion: In former times the transfusion of blood was considered to be a heroic, but in certain cases a very powerful, curative agent. In later times it was thought that physiological salt solution was able to accomplish the same result. On the other hand, our experience makes memorial the phrase "Blut ist ein ganz besonderer saft."

THE STABILITY OF TETANUS TOXINE.

From a practical standpoint it is important to obtain a stable poison. Herein lies the crux of the problem so far as the standardization of tetanus antitoxin is concerned. Tetanus toxine in solution can not be depended upon. Its strength varies so, that if the soluble poisons are used to measure the value of the antitoxic serums, as is the case with the German method, it is found necessary to redetermine by a series of mice the strength of the toxine each time a serum is tested. Diphtheria toxine in solution, after a period of ripening, comes to a stage of equilibrium and after this period is reached retains its toxicity for a long time. This fact is of signal service in the standardization of diphtheria antitoxin. The fact that tetanus toxine does not exhibit the same constancy in solution has thrown much confusion and no little difficulty into the work of standardizing its antitoxin.

Our first efforts therefore were to obtain a stable poison. In this we fortunately succeeded by precipitating the filtered bouillon culture with ammonium sulphate, drying the precipitate over sulphuric acid, grinding to an impalpable powder, and preserving in vacuo under the influence of pentaphosphoric acid—a very strong dehydrating substance—in a cold, dark place. Under these conditions we have found the dried poison maintains its strength without appreciable alteration at least 25 months, as will be seen by the following table:

TABLE No. 4.—*The stability of the standard tetanus toxine A.*

Guinea pig No.	Weight in grams.	Date.	Amount toxine subcutaneously.	Time of death.	
			Gm.	*Days.*	*Hours.*
18	355	Mar. 8,1906	0.000,006	3	9
48	360	Mar. 27,1906	.000,006	3	11
49	350	....do......	.000,006	3	3
63	340	Mar. 31,1906	.000,006	2	10
64	340	....do......	.000,006	3	16
77	325	Apr. 4,1906	.000,006	3	11
78	325	....do......	.000,006	3	7
89	350	Apr. 7,1906	.000,006	3	11
151	335	June 12,1906	.000,006	4	11
152	340	....do......	.000,006	6	9
153	340	....do......	.000,006	5	7
168	320	June 26,1906	.000,006	3	4
199	330	July 26,1906	.000,006	5	9
206	325	Sept. 10,1906	.000,006	3	6
227	350	Nov. 28,1906	.000,006	4	11
228	350	....do......	.000,006	4	6
238	360	Dec. 4,1906	.000,006	3	14
240	365	....do......	.000,006	4	4
245	335	Dec. 7,1906	.000,006	4	6
246	335	....do......	.000,006	7	23
251	395	Jan. 8,1907	.000,006	3	4
252	395	....do......	.000,006	3	12

TABLE NO. 4.—*The stability of the standard tetanus toxine A*—Continued.

Guinea pig No.	Weight in grams.	Date.	Amount toxine subcutaneously.	Time of death.	
			Gm.	*Days.*	*Hours.*
257	305	Jan. 5, 1907	0.000,006	2	14
258	315	.....do.......	.000,006	2	13
263	300	Jan. 7, 1907	.000,006	3	20
264	305	.....do.......	.000,006	4	8
395	395	Feb. 20, 1907	.000,006	5	0
404	315	Feb. 25, 1907	.000,006	3	7
411	340	.....do.......	.000,006	4	4
412	320	.....do.......	.000,006	3	17
434	300	Mar. 22, 1907	.000,006	4	17
435	300	.....do.......	.000,006	3	18
458	300	Mar. 28, 1907	.000.006	4	19
459	300	.....do.......	.000,006	4	18
552	320	May 17, 1907	.000.006	4	19
553	330	.....do.......	.000,066	4	10
593	340	June 25, 1907	.000,006	5	14
604	350	July 12, 1907	.000,006	3	4
609	300	July 20, 1907	.000,006	4	8
610	310	.....do.......	.000,006	5	10
629	370	Aug. 29, 1907	.000,006	4	0
630	390	.....do.......	.000,006	4	8
643	325	Sept. 10, 1907	.000,006	5	15
644	330	.....do.......	.000.006	5	8
658	340	Oct. 11. 1907	.000.006	4	0
659	350	.....do.......	.000,006	4	4
663	350	.....do.......	.000,006	4	0
664	355	.....do.......	.000.006	3	12
703	385	Dec. 19. 1907	.000.006	4	20
704	390	.....do.......	.000.006	5	20
729	330	Feb. 10, 1908	.000.006	4	15
730	340	.....do.......	.000.006	3	19
731	340	.....do.......	.000.006	4	9
749	330	Apr. 7. 1908	.000.006	4	0
750	335	.....do.......	.000,006	3	17
751	335	.....do.......	.000.006	4	2
752	340	.....do.......	.000,006	3	23
753	340	.....do.......	.000,006	3	23

It has fortunately developed that this dried poison not only retains its virulence when kept under the exact conditions above mentioned, but loses its toxicity rather slowly when exposed to light, heat, and other influences. One of the sealed tubes sent from Washington to Manila by mail arrived there without appreciable loss of toxicity.

This dried poison, which is distributed to manufacturers and other laboratories engaged in the work of standardizing tetanus antitoxin, is therefore well suited to withstand transportation. We recommend; however, that it always be kept in a vacuum desiccator in a dark, cold place.

The effect of sunlight upon this toxine will be seen in the following tables:

THE STABILITY OF THE STANDARD TETANUS TOXINE.

In the following experiments the toxine (No. A.) was placed in small wide-mouthed vials, loosely stopped with a pledget of absorbent cotton and exposed under the conditions stated in each table. Table No. 4, page 16, shows the stability of this toxine when kept under special conditions and serves as a control for tables 5 to 8.

Table No. 5.—*Influence of sunlight.*

[MLD=.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
156.....	375	June 23, 1906	6	18	0.000.004	Exposed 54 minutes to direct sunlight and 60 hours to bright light in front of window.
157.....	385	.....do.......	5	19	.000.005	
158.....	400	.....do.......	4	9	.000.006	
159.....	440	.....do.......	4	6	.000.007	
160.....	480	.....do.......	6	8	.000.008	
171.....	320	June 26, 1906	4	2	.000.006	Exposed 54 minutes, again 66 minutes to direct sunlight; and 5½ days bright light in front of window.
172.....	320	.....do.......	4	0	.000.007	
173.....	320	.....do.......	2	20	.000,008	
193.....	310	July 3, 1906	(*a*)		.000,006	Same toxine exposed 4 times, about 1 hour each, bright sunlight and in front of window, 13 days.
194.....	350	.....do.......	(*a*)		.000,007,2	
195.....	370	.....do.......	(*a*)		.000,008,1	
190.....	370	.....do.......	8	21	.000,006	Control. Same toxine kept in vacuum desiccator in dark at 15° C.
191.....	375	.....do.......	6	20	.000,007,2	
192.....	375	.....do.......	5	23	.000,008,1	

[a] Symptoms.

It is evident from the above that two exposures of one hour each to bright sunlight, and five and one-half days to bright light before a window with a southern outlook had no appreciable effect on the dried toxine. However, four exposures of one hour each upon different days to sunlight and thirteen days in bright light slightly diminished the virulence of the poison.

Table No. 6.—*Influence of temperature* 37° *C.*

[MLD=0.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
161.....	375	June 23, 1906	5	20	0.000,004	60 hours at 37° C.
162.....	410	.....do.......	6	1	.000,005	Do.
163.....	480	.....do.......	5	15	.000,006	Do.
164.....	480	.....do.......	6	15	.000,007	Do.
165.....	490	.....do.......	6	6	.000,008	Do.
174.....	320	June 26, 1906	5	1	.000,006	5½ days at 37° C
175.....	320	.....do.......	5	1	.000,007	Do.
176.....	375	.....do.......	4	4	.000,008	Do.

TABLE No. 6.—*Influence of temperature* 37° *C.*—Continued.

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
196	300	July 3, 1906	(a)		0. 000, 006	13 days at 37° C.
197	345	do	(a)		. 000, 007	Do.
198	360	do	(a)		. 000, 008, 1	Do.
217	300	Sept. 25, 1906	(b)		. 000, 006	97 days at 37° C.
218	325	do	(b)		. 000, 007, 2	Do.
219	330	do	(b)		. 000, 009	Do.
220	340	do	(b)		. 000, 009, 9	Do.
221[c]	340	do	2	14	. 000, 012	Do.[c]
222	350	do	(b)		. 000, 012, 9	Do.
223	360	do	(b)		. 000, 014, 1	Do.
224	390	do	(d)		. 000, 015	Do.
225	350	Nov. 28, 1906	3	14	. 000, 004, 2	Control: toxine kept under vacuum at 15° C.
226	350	do	4	4	. 000, 005, 1	Do.
227	350	do	4	11	. 000, 006	Do.
228	350	do	4	0	. 000, 006	Do.
229	350	do	3	16	. 000, 007, 2	Do.
230	350	do	3	19	. 000, 008, 1	Do.

[a] Symptoms.
[b] Slight symptoms.
[c] No tetanic symptoms; died of pneumonia.
[d] Marked symptoms.

An exposure of five and one-half days at 37° C. in the incubator had no apparent effect upon the toxine. After ninety-seven days under the same conditions the poison had lost some of its toxicity.

TABLE No. 7.—*Influence of room temperature, diffuse light, etc.*

[MLD=.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
231		Nov. 30, 1906	(a)		0. 000, 005	163 days in open vial, dark closet, room temperature (June 20 to Nov. 30, 1906).
232		do	11	13	. 000, 006	
233		do	6	18	. 000, 007	
234		do	6	14	. 000, 008	
235		do	6	15	. 000, 009	
236		do	6	23	. 000, 01	
577	320	June 18, 1907	(b)		. 000, 01	363 days in open vial, dark closet, room temperature (June 20, 1906, to June 18, 1907).
578	370	do	(c)		. 000, 02	
579	370	do	(c)		. 000, 04	
580	375	do	5	12	. 000, 06	
581	385	do	4	2	. 000, 08	
582	400	do	3	21	. 000, 1	

[a] Slight symptoms.
[b] No symptoms.
[c] Symptoms.

The standard toxine under these conditions has shown marked stability. After a sojourn of one year (363 days) in a dark closet, at room temperature, it lost in virulence. It will be recalled that the poison was kept in an open vial, loosely stoppered with absorbent cotton, so that it had free access to the oxygen and moisture of the air.

TABLE No. 8.—*Influence of diffuse light, etc.*

[MLD=0.000,006 gram.]

Guinea pig No.	Weight in grams.	Date.	Time of death.		Amount toxine subcutaneously.	Remarks.
			Days.	*Hours.*	*Gram.*	
204	360	July 26, 1906	([a])		0.000,008,1	36 days at room temperature in diffuse light.
205	375	do	4	17	.000,009	
206	375	do	5	17	.000,009,9	
207	375	do	6	3	.000,011,1	

[a] Symptoms.

From this single experiment it will be seen that the toxine exposed to diffuse light at room temperature diminished slightly in toxicity after thirty-six days of such exposure.

It is therefore evident that the dried poison, which we use for the purpose of standardizing tetanus antitoxin, has sufficient stability for practical purposes.

The work of other investigators upon the stability of tetanus toxine and the influence of chemical agents are briefly abstracted in the following:

The first exact work upon the stability of tetanus toxine was done by Kitasato[a] in 1891. This pioneer paper contains an extensive series of tests upon the poison, showing the influence of physical agents and a long list of chemical substances. Kitasato found that the filtrate of tetanus cultures is very sensitive to heat; 65° C. and above is sufficient to destroy it within a few minutes (5 minutes and less). It will sometimes withstand 60°C. for fifteen minutes, but is destroyed at 60° C. for twenty minutes.

It retained its toxic properties when heated an hour and a quarter at 55° C., but lost its power after one and a half hours.

The filtrate of tetanus culture may be dried and retain its toxicity, but this depends upon the manner in which the drying is carried out. In the dessicator over sulphuric acid the filtrate does not lose its properties. The same may be said if it is dried in the air at room temperature, although it is somewhat weakened.

On the other hand, the filtrate was totally destroyed if it is dried in the incubator. The filtrate gradually loses its strength at a temperature of 35° to 37° C.

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[a] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, p. 267–305.

The filtrate placed in the window in diffuse light gradually lost its properties. It lasted, however, a long time before it was entirely destroyed. After it had been nine or ten weeks at the window it was still toxic, but in large doses. The filtrate kept cold in a dark room retained its toxic properties much longer, for after three hundred days it was still equally as toxic as when fresh.

Direct sunlight totally destroys the tetanus poison in from fifteen to eighteen hours.

Diluting the poison with water or bouillon does not seem to harm it in any way.

Hydrochloric acid influences the tetanus poison when added in the proportion of 0.55 per cent within one hour, and in the amount of 0.1365 per cent within twenty-four hours completely destroys the poison.

From similar experiments with many chemical substances Kitasato drew the conclusion that the tetanus poison is very susceptible to acids, especially mineral acids, as well as alkalies.

Tizzoni and Cattani,[a] 1891, have shown that silver nitrate, sublimate, and hydriodic acid alter the toxicity of filtered tetanus cultures; carbolic acid, chlorine water, and iodidum trichloride were also found to have the same effect. Tizzoni and Cattani had much difficulty at first in immunizing animals with large doses of the tetanus toxine in order to obtain potent antitoxin. On account of the virulence of the poison many animals were sacrificed. They found that treating the toxine with iodine trichloride was useful in order to weaken the poison, and thus permit the use of large doses, which could then be rapidly increased.

Vaillard and Vincent,[b] 1891, showed that the tetanus toxine is modified or destroyed by temperatures exceeding 65° C. It is considerably attenuated when heated in a closed flask for forty minutes at 60° C. or twenty minutes at 62° C. Heating for thirty minutes at 65° C. in closed flasks renders it inactive. Kept in a closed flask free from air and light the liquid toxine keeps its activity for a long time; after four months there is no change in its toxicity. Exposed to the air in a thin layer it loses its activity after a month; this action is more rapid and greater in sunlight.

Acidification of the liquid by tartaric acid does not modify its toxicity. It can be dried in a vacuum over H_2SO_4 and is extremely toxic.

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[a] Tizzoni, G., and Cattani, Giuseppina: Ueber die Eigenschaften des Tetanus-Antitoxins. Centblt. f. Bakt., Ref., vol. 9, 1891, pp. 189, 685.

[b] Vaillard, L., & Vincent, H.: Contribution a l'étude du tétanos. Ann. Inst. Pasteur, vol. 5, 1891, p. 1.

Tetanus toxine is insoluble in alcohol. It is precipitated by phosphate of lime or of aluminum; retains its toxicity for a long time; it resists well in this state the action of the air.

Vaillard and Rouget [a] proved that the heating at 65° to 67° C. for five, ten, or fifteen minutes diminishes considerably but does not destroy the toxine. When heated ten or fifteen minutes to 70° or 75° C. and one hour at 80° C. if injected in very large amounts is toxic for guinea pigs. They showed that the spores contained a certain amount of toxine which resisted 80° C. for one hour.

Martin [b] also showed that the toxine is very sensitive to heat; 65° C. or above for five minutes completely destroys it; but it withstands 60° C. for fifteen minutes and 55° C. for an hour and a quarter; much longer at lower temperatures. He dried it over H_2SO_4 without loss of toxicity. If dried in the incubator it loses its toxicity. It was destroyed in nine or ten weeks by diffused daylight; fifteen to eighteen hours direct sunlight completely destroys it.

Lumière and Chevrottier [c] found that solutions of permanganate of potash and sulphate of iron, and the salts of cerium, cobalt, and nickel markedly attenuate the toxine of tetanus.

Fermi and Pernossi [d] draw the following conclusions from their studies of the tetanus poison:

Agar cultures are the most poisonous. Next come those on gelatin, and lastly those in bouillon. Chickens, snakes, turtles, and tritons are immune to the poison. In the above-mentioned animals this toxine may remain and retain its virulence for three days, and even longer. Filtrates from agar and gelatin cultures are more resistant to heat than those from bouillon. Like the enzymes, the purer the tetanus poison the less stability does it possess. Dissolved in water, the tetanus poison is rendered inert by a temperature of 55° C., but in the dry state it can be heated to 120° C. without loss of virulence. When the dry poison is mixed with ether or chloroform and heated to 80° C. it is destroyed; but with amylic alcohol or benzol a temperature of 100° is required to accomplish this result. Dissolved in water this poison is destroyed by direct sunlight after an exposure of eight to ten hours (with the highest temperature on a blackened thermometer at 56° C.) and after fifteen hours when the temperature does not exceed 37° C. In the dry state the tetanus poison can be exposed to the direct sunlight for one hundred hours without loss of virulence. Under the action of an electric current of 0.5 ampere, continued for two hours, the substance becomes inert. Gastric juice destroys the poison through the activity of the hydrochloric acid and not by virtue of the pepsin. Ptyalin, diastase, and emulsin have no action on it. The effect of trypsin has not been satisfactorily determined. Putrefactive germs do not destroy the poison. The living, but not the dead, intestines of guinea pigs and cats destroy its toxicity. The poison may be eliminated by the kidneys and retain its properties in the urine; it is not a ferment.

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[a] Vaillard, L., & Rouget, J.: Contribution a l'étude du tétanos. Ann. Inst. Pasteur, vol. 6, 1892, p. 385.

[b] Martin, S.: Further report on the chemical pathology of tetanus. Report of med. officer, Local Govt. Board, London, 1894–5, vol. 24, 1896, pp. 505–513.

[c] Lumière, A. & L., & Chevrottier, J.: Action des oxydases artificielles sur la toxine tétanique. Compt. rend. acad. sci., vol. 138, 1904, pp. 652–654.

[d] Fermi, Cl., and Pernossi, Leone: Ueber das Tetanusgift. Vergleichende Studien mit Berücksichtigung anderer, Gifte und der Enzyme. Centblt. f. Bakt., 1 Abt., vol. 15, 1894, pp. 303–310. Ref., Vaughan & Novy's "Cellular toxins," 1902, p. 62–63.

Ritchie [a] states that tetanus toxine under the influence of hydrochloric acid loses with comparative readiness its virulently poisonous properties. It does not, however, so readily lose its capacity of producing immunity, and when all trace of toxicity has disappeared the capacity of producing immunity still remains. The less poisonous substances produced in the modified toxine are probably of the nature of toxoids.

Tetanus toxine is also susceptible to the action of alkalies, such as sodium hydrate and sodium carbonate, under which it loses its toxicity.

Noguchi,[b] 1907, found that eosin, if present in cultures containing tetanus spores, prevents the germination of these spores when its concentration (in glucose bouillon) reaches 0.2 per cent.

The toxine production of tetanus bacilli grown in eosinized culture media diminishes as the concentration of the eosin increases. This effect is brought about partly by the restraining action of the dye on vegetation and partly by its detoxicating action upon the poison.

The toxine-producing power and the virulence of tetanus bacilli are not permanently modified by contact with eosin for a long period or by successive cultivations in eosinized media.

Burckhard[c] found that formalin when added to tetanus toxine in the proportion of 1 to 250 was sufficient to protect a mouse against a dose of tetanus toxine which caused the death of the control in twenty-four hours. Burckhard does not seem to have determined the fatal dose of his tetanus toxine.

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[a] Ritchie, James: Artificial modifications of toxins with special reference to immunity. Journ. hygiene, vol. 1, 1901, pp. 125–144.

[b] Noguchi, Hideyo: The nature of the antitetanic action of eosin. Journ. exper. med., vol. 9, no. 3, May 25, 1907, pp. 281–290.

[c] Burckhard, G.: Zwei Beitrage zur Kenntniss der Formalinwirkung. Centblt. f. Bakt., 1 Abt, vol. 18, 1895, pp. 257–264.

One of us (Anderson),[a] 1907, found that when tetanus toxine is exposed to 5 per cent formalin for six hours a guinea pig is able to withstand 100 MLD of this formalinized toxine. Three per cent formalin after twenty-four hours' exposure protects against the toxine; it destroys a part of the toxine in one hour, its action increasing with the length of exposure.

THE TETANUS TOXINE.

On account of its virulence, its solubility, and the characteristic contractions which it induces the poison of tetanus has been a convenient and favorite subject of investigation. It was the first of the bacterial toxines to give fruitful results in the realm of serum therapy.

Tetanus toxine is readily soluble in the medium in which the culture grows whether fluid or solid. We shall presently see that the filtered culture, called the "tetanus toxine," is really a complex substance, containing various poisons and other bodies.

The particular poison that concerns us especially is the *tetano-spasmine,* which produces the convulsions characteristic of the disease. This poison is a type of a true toxine. It is readily rendered inert by heat, contains both a toxophore and a haptophore group, induces antibodies when introduced into susceptible organisms, and produces symptoms only after a definite period of incubation. Its poisonous action is destroyed by the digestive juices. In all these characteristics tetanus toxine resembles diphtheria toxine. It is one of the most poisonous substances known. As small an amount as 0.000,006 gram of our standard precipitated toxine invariably kills a guinea pig weighing 350 grams. As the precipitate consists mostly of albumins, peptone, amido acids, volatile substances, ammonium sulphate, and other salts it will be seen that but a small proportion of the weight consists of pure poison. Our standard toxine is by no means as strong as other tetanus poisons that have been prepared.

Brieger and Cohn[b] found their strongest tetanus poison killed mice weighing 15 grams when given subcutaneously in doses of 0.000,000,05 gram; smaller doses, such as 0.000,000,01 gram, caused more or less tetanic symptoms in mice. If 0.000,000,05 gram kills a mouse weighing 15 grams Brieger and Cohn calculate that 0.000,23 gram would be a fatal dose for a man weighing 70 kilograms.

Tetanus toxine is not equally toxic for all species of animals. On the other hand, there is an extraordinary constancy in its toxicity upon individuals of the same species. The same quantity of poison per gram weight of a particular animal always produces similar results.

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[a] Anderson, John F.: The antiseptic and germicidal properties of solutions of formaldehyde and its action upon toxines. Hyg. Lab. Bull. No. 39, U. S. Pub. Health and Mar. Hosp. Serv., Wash., 1907, pp. 47. 8°.

[b] Brieger, Ludwig, and Cohn, Georg: Untersuchungen das Tetanusgift. Zeit. f. Hyg., vol. 15, 1893, pp. 1–10.

In 1893 Wladimiroff [a] conducted under Behring's direction a series of experiments to determine the minimal fatal dose for various species of animals. Considering the susceptibility to tetanus toxine for mice as 1, we have the following results. The minimal fatal dose is calculated to the body weight:

Animal species.	Minimal fatal dose.	Susceptibility.
White mice	1 : 500,000	1
White rats	1 : 50,000	0.1
Guinea pigs	1 : 1,000,000	2
Rabbits	Greater than 1 : 24,000	Considerably less than $\frac{1}{20}$.
Goats	1 : 250,000	About $\frac{1}{2}$.

Expressed in figures of minimal lethal doses for the above-mentioned animals we have the following:

Animal species.	Minimal fatal dose.	Minimal fatal dose expressed in minimal fatal dose for mice.
White mice	0.00004	1
White rats	0.004	100
Guinea pigs	0.0002	5
Young goats	0.1	25.000
Medium size rabbits	5.0	125,000

According to Behring, 1 minimal lethal dose per mouse is equivalent to 12 for a horse, 6 for a guinea pig, $\frac{1}{2}$ for a goat, $\frac{1}{150}$ for a rabbit, $\frac{1}{1000}$ for a goose, $\frac{1}{4000}$ for a pigeon, and $\frac{1}{30000}$ for a chicken. That is, the horse is 12 times as susceptible as the mouse, and the mouse is 30,000 times more susceptible than the chicken.

Knorr [b] in his paper on the subject gives a scale of susceptibility to tetanus poison. He finds the horse to be the most susceptible animal. If we take the dose that kills 1 gram of horse as unity, the amounts of poison required by the following animals is as follows:

1 gram of guinea pig	2 times as much
1 gram of goat	4 times as much
1 gram of mouse	13 times as much
1 gram of rabbit	2,000 times as much
1 gram of hen	200,000 times as much

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[a] Wladimiroff, A.: Ueber die Antitoxinerzeugende und immunisirende Wirkung des Tetanusgiftes bei Thieren. Zeit. f. Hyg., vol. 15, 1893, pp. 405–422.

[b] Knorr: "Das Tetanusgift und seine Beziehg. zum tier. Organismus," Münch. med. Woch., 1898, 321, 362.

There is always a period of incubation in the animal organism. The duration of this period varies with the species. As a rule, the period of incubation is shorter the more susceptible the animal and the higher the dose. According to Behring:

	Incubation (hours).
13 lethal doses per gram, mouse	36
110 lethal doses per gram, mouse	24
333 lethal doses per gram, mouse	20
1,300 lethal doses per gram, mouse	14
3,600 lethal doses per gram, mouse	12

The period of incubation is never shorter than eight hours.

We have found in the guinea pig that the period of incubation varies inversely with the size of the dose; that is, within certain limits the larger the dose the shorter the period of incubation. This period may vary from about eighteen hours to about five days.

We have found that there is a direct relation between the period of incubation and the severity of symptoms in guinea pigs. Guinea pigs that show symptoms within forty-eight hours after injections of tetanus toxine (either M. L. D. or L+) invariably die. Guinea pigs that show symptoms on the third day usually die, a very small percentage recovering. The longer the onset of symptoms is delayed, the milder the disease and the greater the chances of recovery. These statements are based upon a tabulation of 600 serial cases.

Tetanus toxine is harmless when given by the mouth. It is not absorbed from the intact intestinal tract and is destroyed by the digestive juices.

As a result of work upon guinea pigs, Ransom[a] concluded that tetanus toxine, even in very large doses, is harmless so long as the intestinal tract is intact; that the poison is not absorbed either from the stomach or from the intestines, and in consequence neither the poison nor its antitoxine appears in the blood; and, finally, that the poison is not destroyed in the intestinal canal, but flows through the entire canal and is thrown off *per anum*.

Carrière, however, and several other investigators were unable to detect any toxine in the excreta after the introduction of large doses of toxine by the mouth.

Carrière[b] accordingly made experiments to determine where tetanus toxine became innocuous. He found that the poison was attacked even by the saliva diastase, that pepsin was less injurious, that trypsin had considerable action upon it, and that bile in large quantity completely destroyed it. He could not detect any influence of the intestinal mucous membrane and the intestinal bacteria upon the poison, although Fermi and Pernossi had found it to be very pronounced. Nencki and Schoumow-Simanowski[a] assert that the digestive fluids render it completely innocuous, notably the bile in conjunction with the fluid from a pancreatic fistula, while trypsin alone has less effect, being less injurious than pepsin. Vincenzi,[b] on the other hand, states that *normal* bile has hardly any destructive influence, but that the bile of animals infected with tetanus has a slight effect under certain conditions; thus, for example, death inevitably follows in three to four days when the bile is active. On the other hand, the poison is somewhat weakened by the *oxydase* of the leucocytes, and, according to Sieber,[c] is completely destroyed by the oxydase of the spleen.[d]

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[a] Ransom, F.: Das Schicksal des Tetanusgiftes nach seiner intestinalen in den Meerschweinorganismus. Deu. med. Woch., vol. 24, 1898, pp. 117–118.

[b] Carrière: "Toxines et digestion." Ann. Past., xiii, 435, 1899 (gives a bibliography of the subject), cf. the General Part.

We have fed guinea pigs as much as 24,000 and mice 18,000 times the M. L. D. of tetanus toxine (if injected subcutaneously) without producing any apparent ill effects.

Up to the present time all attempts to isolate the specific toxine as a definite chemical compound have proved unavailing; we are totally ignorant of its chemical nature. Animal experiments are the only real agents by which it may be recognized. By this means we are enabled to determine not only the presence of the poison, but also to estimate its concentration in a solution and to follow its changes.

Before the tetanus bacillus was obtained in pure culture the chemical nature of the toxine was studied by Brieger from mixed cultures in meat broth obtained from Nicolaier and Rosenbach. In 1886 Brieger[e] isolated from such a contaminated growth a basic substance or ptomaine which he called *tetanine* ($C_{13}H_{30}N_2O_4$), and which caused muscular contractions when injected into mice.

Shortly afterward Brieger[f] obtained another ptomaine which he named *tetano-toxine* ($C_5H_{11}N$). He believed these to be the true poison of tetanus. These are now only of historical interest.

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[a] Nencki and Schumow-Simanowski: "Ueber die Entgiftung der Toxin durch die Verdauungssäfte." Centralb. f. Bakt., xxiv, 84, cf. Dzierzgowski and Sieber. Archiv des Siences Biol. de St. Petersb., viii.

[b] Vincenzi: "Ueber antitoxische Eigenschaften der Galle tetanisierter Tiere." Deutsch. med. Woch., 1898, 534.

[c] Sieber: "Ueber d. Entgiftung der Toxin durch die Superoxyde, etc." Z. f. phys. Ch., xxxii, 573, 1901.

[d] Toxines and Antitoxines; Oppenheimer, Carl, trans. by C. Ainsworth Mitchell. Charles Griffin & Co., Ltd., London, 1906, p. 110.

[e] Brieger, L.: Ueber ein neues Krämpfe verursachendes Ptomain. Berichte d. deut. chem. Gesellsch., vol. 19, Dec. 9, 1886, p. 3119.

[f] Brieger, L.: Zur Kenntniss der Aetiologie des Wundstarrkrampfes, nebst Bemerkungen über das Choleraroth. Deut. med. Woch., vol. 13, 1887, p. 303.

———: Ptomaine. 3 bd., 1886, p. 89.

———: Ueber ein neues Krämpfe verursachendes Ptomain. Berichte d. deut. chem. Gesellsch., vol. 19. 1886. p. 3119.

Knud Faber,[a] in 1890, first succeeded in demonstrating by physiological tests upon animals the poison in the filtrate of mixed cultures; but exact work upon the toxine dates from Kitasato's anærobic method, in 1889 and 1891, of growing the tetanus bacillus in pure culture upon solid and fluid media.

Brieger's experiments were repeated by Kitasato and Weyl[b] in 1890 with pure cultures of tetanus. From their studies they concluded that Brieger's tetanin, etc., did not produce the characteristic symptoms of tetanus in experimental animals.

Brieger and Fränkel,[c] in 1890, in further experiments believed the tetanus toxine, as well as the poisons produced by the whole order of important pathogenic micro-organisms, to be "toxalbumins." Brieger and Fränkel's toxalbumin consisted practically of an alcoholic precipitate from filtered broth cultures and was undoubtedly toxic.

Hayashi[d] concludes, from his work upon the subject, that the toxine isolated according to the Brieger-Boers method, as well as by his own modification, shows a definite albumin reaction. However, this is not proof that the toxine is a proteid.

The powerful action of tetanus poison in minute amounts, its thermolability, and the period of incubation lend countenance to the view that the toxine may be a ferment. But ferments continue to act when the products of fermentation are removed. This has not been shown to be the case with tetanus toxine. Many of the higher albuminous substances are also readily destroyed by heat lower than 65° C., which is fatal for the tetanus toxine. The period of incubation is now explained by the time necessary for the poison to travel up the motor nerves to the centers. There is, therefore, nothing but analogy of an indefinite nature to class tetanus toxine with the ferments.

Ehrlich,[e] in a parallel work to his researches upon the constituents of the diphtheria toxine, showed that the tetanus toxine contains both a toxophore and a haptophore group, and that the antitoxic immunity is explained by the presence of free receptors in the blood of immunized animals. The receptors combine directly with the haptophore group, thus neutralizing the toxine.

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[a] Feber, Knud: Die Pathogenese des Tetanus. Berl. klin. Woch., vol. 27, 1890, pp. 717–720.

[b] Kitasato, S., and Weyl, Th.: Zu Kenntniss der Anaeroben. Zeit. f. Hyg., vol. 8, 1890, pp. 41, 404.

[c] Brieger, L., and Fränkel, Carl: Untersuchungen über Bakteriengifte. Berl. klin. Woch., vol. 27, 1890, p. 268.

[d] Hayashi, H.: Weitere Forschungen über die chemische Natur des Tetanustoxins. Arch. exper. Path. u. Pharm., vol. 47, 1901–1902, pp. 9–18.

[e] Ehrlich, P.: Die Werthbestimmung des Diphtherieheilserums. Klin. Jahrb., 1897.

———: Ueber die Constitution des Diphtheriegiftes. Deut. med. Woch., vol. 24, 1898.

Ehrlich,[a] 1898, definitely proved that tetanus toxine contains at least two poisons—(1) *tetano-lysin* and (2) *tetano-spasmin*. He showed that these two poisons do not always appear in the same relative proportion in different preparations. Some of the toxines that have strong tetanic properties have weak hemolytic action, and *vice versa*. The hemolytic affinity of the toxine weakens much quicker than the tetano-spasmin. This occurs spontaneously as well as when it is heated to 50° for twenty minutes. The two poisons have different combining affinities. If tetanus toxine is brought into contact with red blood corpuscles, the greatest part of the tetanolysin is bound by the red corpuscles, while the tetanospasmin remains in the solution. Each one of these two poisons has its own antitoxin. If several different tetanus sera are examined, it will be found that they have no parallel neutralization for tetanolysin and tetanospasmin. In one particular case Ehrlich found a serum that was strongly antispastic and had practically no antilytic power.

Madsen,[b] 1899, confirms Ehrlich's observations that in cultures of the tetanus bacillus there are two poisons, namely, tetanolysin and tetanospasmin, and that for the former there exists a specific antitoxin, namely, antilysin. The strength of this lysin and its antibodies may be measured with great accuracy by colorimetric tests.

Knud Faber,[c] in his early work with mixed cultures in 1890, was the first to obtain indications of the true tetanus poison.

Faber showed that the poison always works after a certain period of incubation. The greater the quantity of poison the shorter the period of incubation and the shorter the course of the disease. For instance, when the period of incubation was twenty-four hours, rabbits died after a sickness of twenty-four hours; but if the period of incubation was five days, then the rabbits recovered after localized tetanus lasting fourteen days. With mice the period of incubation varied from six hours to two to fifteen days.

He found that the poison is active not only when injected into the tissues, but also when injected intravenously in rabbits. Positive results were obtained with mice, rats, guinea pigs, rabbits, and birds, but frogs were not affected.

He was not able to obtain an immunity by using the filtered cultures. The poison passes through a Chamberland filter. All traces of toxicity disappear if the poison is heated for five minutes at 65° C. Faber states that experimental tetanus in smaller animals always manifests itself by local cramps. This is the case whether the toxine or a bacterial culture is used; so that we must conceive that the disease spreads in and around the wound, working upon the motor nerve endings (?). The general spasms are produced by the introduction of the poison into the blood.

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[a] Ehrlich, P.: Gesellschaft der Charité-Aertze (Sitzung 3. Februar 1898). Berl. klin. Woch., 1898, no. 12.

[b] Madsen, Thorvald: Ueber Tetanolysin. Zeit. f. Hyg., vol. 32, 1899, pp. 214, 239.

[c] Faber, Knud: Die Pathogenese des Tetanus. Berl. klin. Woch., vol. 27, 1890, pp. 717–720.

In 1891 Kitasato,[a] working under Koch's directions, published his classical paper upon tetanus toxine. The toxine was readily obtained in any desired amount by simply filtering pure cultures of the organism.

Of the three species of animals used in these experiments—mice, guinea pigs, and rabbits—Kitasato found that guinea pigs are most susceptible to tetanus toxine; then follow mice, and finally rabbits. The symptoms of tetanus in all three animals injected with the filtrate appear at the latest in three days. If the animals remain well for four days they will not sicken with tetanus.

The tetanus toxine appears in the blood and there produces its toxic properties. The blood itself is sterile, and therefore it is a toxic and not a bacterial result. The bouillon must be neither acid nor strongly alkaline, but about neutral or have a weak alkalinity; and, secondly, it must always be freshly prepared.

Meyer[b] injected dogs and cats with tetanus toxine into the spinal cord and found the condition known as tetanus dolorosus. This is never found in any other method of tetanus poisoning. By injecting the posterior roots of the spinal cord in the lumbar region Meyer obtained a pure tetanus dolorosus without muscular contractions.

Bolton and Fisch[c] have shown that the toxine makes its appearance in the blood of the horse several days before any symptoms of tetanus are to be observed, and that it gradually increases until about two days before symptoms become noticeable, and then it suddenly diminishes, and even disappears in some cases. The amount of toxine varies considerably in different cases. In one instance the serum from a diphtheria antitoxin horse about two days before symptoms of tetanus appeared was sufficient to kill a guinea pig in the dose of 0.1 c. c. The fact that tetanus toxine may appear in such large quantities in the blood without symptoms of tetanus is of very great practical importance in the production of both diphtheria and tetanus antitoxins.

Von Lingelsheim[d] recommends that in order to obtain strong tetanus toxines only those cultures which are strictly anaërobic should be used. According to Debrand,[a] the strongest poisons may be obtained by symbiosis with *B. subtilis.* The best medium is a weak alkaline bouillon with 1 per cent peptone and 0.5 per cent salt. The addition of glycerin and grape sugar is to be avoided, as the acids produced by their decomposition have an injurious effect upon the toxine. Tizzoni obtained good results by the addition of gelatin to the culture medium. It is most important to have a culture which is a good toxine producer, just as in the case of diphtheria. It seems that the strongest poisons are obtained after a term of from six to eight days in the incubator. By observing the proper conditions it seems comparatively easy to obtain cultures that will kill a mouse weighing 15 grams in a dose of 0.000005 gram. Most of those who have worked much in this subject have experienced at times difficulty in obtaining strong tetanus toxine; not all of the factors involved are understood.

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[a] Kitasato, S.: Experimentelle Untersuchungen über das Tetanusgift. Zeit. f. Hyg., vol. 10, 1891, pp. 267–305.

[b] Meyer, Hans: Ueber motorischen und dolorosen Tetanus. Sitzber. d. Gesellsch. z. Bef. d. Naturw., Marburg, 1902, pp. 1–6.

[c] Bolton, B. Meade, and Fisch, Carl: An estimate of the amount of toxine in the blood of horses infected with tetanus. Trans. Assn. of Am. Phys., vol. 17, 1902, pp. 462–467.

[d] Von Lingelsheim, W.: Das Tetanusgift. Kolle & Wassermann's Handbuch der Bakterien, Bd. 2, p. 589.

It is noteworthy that the strength of the toxine produced is greater in contaminated cultures than in pure cultures. In the Marburg Institute of Hygiene and Experimental Therapy, presided over by Behring,[b] the strongest tetanus toxines have been obtained from mixed cultures. The first work, it will be remembered, upon the tetanus toxine by Faber, Brieger, Fraenkel, Nicolaier, and Rosenbach was with impure cultures.

Uschinsky[c] cultivated tetanus bacilli on a proteid-free medium of the following composition:

Water	1,000
Glycerin	30 to 40
Sodium chloride	5 to 7
Calcium chloride	0.1
Magnesium sulphate	0.2 to 0.4
Dipotassium hydrogen phosphate	2 to 2.5
Ammonium lactate	6 to 7
Sodium aspartate	3.4

preferably with the addition of 1 to 2 per cent of grape sugar. The air was excluded by means of liquid paraffin.

According to Buchner,[d] Tizzoni and Cattani, following the plan of Brieger and Fränkel, were the first to succeed in obtaining a dried poison with the use of ammonium sulphate.

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[a] Debrand, L.: Sur un nouveau procédé de culture du bacille du tetanos. Ann. d. Inst. Pasteur, vol. 14, 1900, pp. 757–768.

[b] v. Behring, E.: Aetiologie und aetiologische Therapie des Tetanus. Beitr. z. exper. Therapie, vol. 7, 1904, pp. 1–72.

[c] Uschinsky, "Ueber eine eiweissfreie Nährlosung f. pathog. Bakt." Cantralb. f. Bakt., vol. 14, 1893, p. 316.

[d] Buchner, H.: Erwiderung betreffend das trockene Tetanusgift. Deut. med. Woch., vol. 20, 1894, p. 179.

Brieger and Fraenkel,[a] however, call attention to the fact that in 1890, in the Berliner klinische Wochenschrift, No. 11, page 612, they describe a process for obtaining a dried tetanus poison and experiments with the same.

Whoever owns priority, the method is a very good one, and with it we have succeeded in obtaining strong and stable poisons.

MODE OF ACTION OF TETANUS TOXINE.

For a full understanding of the problem with which we are dealing it is important to have a clear conception of the way in which the tetanus toxine exerts its poisonous effects upon the body. Therefore the following review of the literature upon this question has been introduced.

It has been known since ancient times that tetanus toxine affects chiefly the central nervous system, but it is only comparatively recently that it has been experimentally demonstrated in what way the toxine reaches the nervous centers.

Gumprecht[b] first showed with some degree of certainty that the toxine attacks particularly the spinal cord and that the phenomenon of the disease depends upon this cord poisoning.

Bruschettinni,[c] 1892, demonstrated the presence of the toxine in the central and peripheral nervous system after subcutaneous inoculation. He also showed it to be present in the blood, but that the other organs were free from it.

Brunner,[d] 1892, in experiments upon guinea pigs and rabbits, found that the tetanus poison was not able to cause the tetanic contractions when placed directly upon the muscle substance. After paralysis of the motor nerve terminals by curare the tetanus contractions ceased. He found, however, that the tetanus toxine is not able to cause spasms in a muscle area the motor nerve of which has been severed from its center. In view of the first fact, it followed that a motor nerve severed from its center is not able to produce tetanus contractions.

Brunner further concluded that the poison was able to induce tetanic spasms in the muscles of a particular nerve area only when the paths to the corresponding nerve centers are intact.

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[b] Brieger, Ludwig, and Fraenkel, Carl: Herr Buchner und das trockene Tetanusgift. Deut. med. Woch., vol. 20, 1894, p. 118.

[b] Gumprecht: Versuche über die physiologische Wirkung des Tetanusgiftes im Organismus. Arch. f. Physiol.

[c] Bruschettinni, Alessandro: Sulla diffusione del veleno del tetano nell'organismo. La riforma med., vol. 8, part 3, 1892, pp. 256–259.

[d] Brunner, Conrad: Zur Pathogenese des Kopftetanus. Berl. klin. Woch., 1891.

———: Die bisherigen Resultate experimenteller Untersuchungen über die Art der Wirkung des Tetanusgiftes auf das Nervensystem. Deut. med. Woch., vol. 20, 1894, pp. 100–103.

Vaillard and Vincent,[a] 1890, had already shown that the contractions in a particular innervation of the area disappeared when such an area was cut off by section of the cord.

Knorr,[b] 1897, as a result of his work, concluded that:

> Pure tetanus poison has a strong affinity for some unknown substance in certain of the body cells. The amount of this affinity is as the degree of susceptibility of animals (that have not previously been treated) for the poison.
>
> The antitoxin has the same property of combining with the toxine as has the abovementioned unknown substance.
>
> In the culture media of tetanus bacilli there are also sometimes found bodies that have an affinity for the poison.
>
> The combination of the poison with all of these substances is slow. The rapidity depends upon the degree of the affinity and the concentration, and other conditions surrounding the poison.
>
> For every animal species the dose most favorable for the production of antitoxin seems to be slightly less than that necessary to make the animal sick. Doses that are much smaller are not effective. Doses that are considerably higher interfere with the production of antitoxin. The production of antitoxin begins very soon after the inoculation of a favorable amount of toxine.
>
> The antitoxin is a product of the body and appears to be a specific substance of the cells.

The tetanus toxine combines chemically, according to Milchner,[c] 1898, with the antitoxic group of the central nervous system in the test tube, without the help of the living organism. This indicates, according to Ehrlich's theory, that there is also a chemical union between the toxine and the nervous system in the living body.

Marie,[d] 1898, showed that the toxine injected into animals remains a variable time in the blood; this time passed, the inoculation of the organs and glandular secretions is negative. When injected under the skin, a part of the toxine is taken up by the nerve fibers. He also found that the brain of rabbits neutralizes tetanus toxine. The cells of the central ganglions are much less active than those from the cerebral surface.

For the purpose of testing the action of cord and brain of normal animals on tetanus toxine, Wassermann and Takaki,[e] 1898, used a solution of tetanus toxine, containing as a preservative an equal amount of glycerin, and of such strength that 0.001 c. c. was a fatal dose to a mouse weighing 15 grams within three days after inoculation.

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[a] Vaillard and Vincent: Sur. le poison tétanique. La sem. med., vol. 10, 1890, pp. 425–426.

[b] Knorr, A.: Die Entstehung de Tetanusantitoxins im Theirkörper und seine Beziehung zum Tetanusgift. Fortschr. d. Med., vol. 15, 1897, pp. 657–669.

[c] Milchner, Richard: Nachweiss der chemischen Bindung von Tetanusgift durch Nervensubstanz. Berl. klin. Woch., vol. 35, 1898, pp. 369–371.

[d] Marie, A.: Recherches sur les la toxine tétanique. Ann. Inst. Pasteur, vol. 11, 1897, p. 591; vol. 12, 1898, p. 91.

[e] Wassermann, A., and Takaki, T.: Ueber tetanusantitoxische Eigenschaften des normalen Centralnervensystems. Berl. klin. Woch., vol. 35, 1898, pp. 5–6.

They mixed a single, double, triple, ten times fatal dose, etc., of the tetanus toxine with 1 c. c. of emulsion of normal cord or brain in a flask and injected this subcutaneously in the dorsal region. As controls, equal amounts of emulsion of liver, kidney, spleen, marrow, or 1 c. c. serum of same animal were mixed with the specified quantities of toxine and likewise subcutaneously injected into mice, and as a further control the tetanus toxine alone was injected. In all their experiments thus carried out they could, without exception, demonstrate that normal brain or cord always had an antitoxic action, while no other tested organ showed this action.

The antitoxic power of the brain of normal guinea pigs is so decided that 1 c. c. of brain emulsion will neutralize ten times the lethal dose. A definite retarding influence is exerted by the emulsion on a 60-times lethal dose. The cord is definite, though feebler, in its influence; its emulsion will neutralize three times the fatal dose.

They were able to immunize against three and five times the fatal dose by an injection of emulsion of brain twenty-four hours before. They could even save a mouse that had received a fatal dose of toxine by the injection of the emulsion four to six hours after. The tetanus toxine-binding power of the central nervous system acts not only by direct mixing in vitro, but also in the living organism. Their experiments lead them to believe that the fixing power of the central nervous system is due to a property of the cell and not to a water-soluble substance.

Metchnikoff,[a] 1898, agrees with Wassermann and Takaki that the brain and cord of guinea pigs neutralize tetanus toxine. From his own experiments he concludes that the antitetanic action of the nervous centers is a property of mammals. The nervous centers of the fowl are much less efficacious, the tortoise only very feebly so, and the frog not at all.

Roux and Borrel,[b] 1898, find that animals are much more sensitive to intracerebral than to subcutaneous injections of toxine. Animals highly resistant to injections under the skin or in the veins are sensitive to injections into the brain. The nerve cells are still sensitive to the toxine.

Danysz,[c] 1898, showed that:

> The cerebral substance of guinea pigs fixes a variable amount of tetanus toxine according to the medium used.
>
> The quantities of toxine fixed by the cerebral substance are much greater when the liquid itself in which the emulsion has been made, exercises the least energetic modify-

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[a] Metchnikoff, E.: Sur l'influence de l'organisme sur les toxine. Ann. Inst. Pasteur, vol. 12, 1898, p. 81.

[b] Roux, E., and Borrel, A.: Tétanos cérébral et immunité contre le tétanos. Ann. Inst. Pasteur, vol. 12, 1898, p. 225.

[c] Danysz: Contribution a l'étude de la toxine tétanique. Ann. Inst. Pasteur, vol. 13, 1898, pp. 156–168.

ing action on the toxine. This quantity is the greatest in physiologic salt solution, which best preserves the cerebral substance in its normal state. If the amount fixed by the brain emulsified in normal salt solution be taken as 100, we will have for the others about the following proportions:

If a brain of a guinea pig emulsified in normal salt solution absorbs 100 parts toxine, the same brain emulsified in distilled water will absorb 90 parts toxine; if emulsified in 10 per cent salt solution, will absorb 5 parts toxine; if boiled in distilled water, will absorb 10 parts toxine.

The fixation of the toxine by the cerebral substance is not permanent, as has been previously shown by Metchnikoff. It diffuses in the liquid of maceration, and this diffusion is much more rapid when the liquid exercises a more energetic action upon the nervous substance.

He concluded that the phenomenon of the fixation of the tetanus toxine by nervous tissue, in spite of some analogies, can not be likened to the action of antitoxin on toxine, because the toxine at first fixed by the nervous substance becomes again free *in vitro* and *in vivo.* The neutral mixture of toxine and nervous substance becomes again with time more and more toxic, while, on the contrary, the toxic mixture of toxine and antitoxin become with time less toxic.

Blumenthal and Jakob,[a] 1898, used a method to free a part of the tetanus toxine which has been fixed in the central nervous system. They concluded that the tetanus poison at the time of the outbreak of the symptoms of tetanus is so anchored in the central nervous system that it can not be set free by means of subdural infusions of antitoxin.

Ransom[b] found that the tetanus toxine as well as the antitoxin is taken up from the subcutaneous tissue by means of lymphatic vessels and carried to the blood stream.

After intravenous injection a considerable portion of the toxine as well as the antitoxin is carried over into the lymph. In the cerebrospinal fluid, on the other hand, the antitoxin appears in comparatively small amounts and the toxine can not be definitely demonstrated. Tetanus antitoxin injected into the subarachnoid space does not cause an increase in the normal, detoxicating power of the nerve substance and most all of it quickly passes over into the blood stream.

After injections of the toxine into the central nervous system the nervous system becomes toxic itself and loses for a time the property of neutralizing the poison. This is not due to adherent cerebro-spinal fluid, but rather to the fact that the nerve substance holds the toxine fast.

The disappearance of tetanus toxine from the blood of chickens after intravenous injections of small quantities does not depend upon the combination of the unchanged toxine in any one organ. Up to the time when the toxine disappears and the antitoxin begins to appear in the blood no collection of antitoxin can be found in any organ. The intravenous injection of very small quantities of toxine into hens, quantities too small to cause harmful reactions, causes considerable antitoxin to appear.

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[a] Blumenthal, Ferdinand, and Jakob: Zur Serumtherapie des Tetanus. Berl. klin. Woch., 1898.

[b] Ransom, F.: Die Vertheilung von Tetanusgift und Tetanusantitoxin in lebenden thierischen Körper. Berl. Klin. Woch, vol. 38, 1901, pp. 337–340; 373–375.

The above experimental work on the affinity of the nerves for the toxine and the neutralization of the toxine by the nervous tissue led Marie and Morax,[a] 1902, and Meyer and Ransom, 1903, independently to study the method of absorption of the toxine. The paper by Marie and Morax was published in 1902. They find that the absorption of tetanus toxine by the peripheral nerves is in consequence of the specific affinity for the axis cylinder substance. This affinity is not shown in experiments *in vitro,* contrary to those which are observed with the cord or with the brain, but is shown with the greatest ease by experiments *in vivo.* Fixation of the toxine upon the nerves occurs very rapidly. It has the characteristic of not being very stable.

They showed that the toxine once fixed, is displaced centripetally with a relatively feeble rapidity. This axis cylinder circulation has for its effect the transportation of the diluted toxine in the fluids to the ganglionic cells. The authors believe that they are able to formulate the following hypothesis: Injected into a muscle or into a region a little removed from a muscular mass, the toxine distributes itself in the serous fluid which impregnates the tissues, and passes in part to the blood, where it can be found very soon. In the zone of inoculation the serous fluid charged with the toxine is found in contact with expansions of the nerves at their endings. The motor nerves as well as the vaso-motor nerves absorb it and transfer it, so that in a relatively short time the substance of the peripheral nerves contains it in quantity larger than the fluids which bathe the tissues at some distance from the point of absorption. Diffusion of the toxine occurs centripetally. It may be compared to the absorption of nourishing liquids by the roots of a plant. The portion of toxine contained in the lymph at the point of contact remains twenty-four hours or less. This amount is greater than the portion contained in the blood, and consequently in the lymph in different parts of the body. Absorption by the nerve fibers at the region inoculated is then greater during the first twenty-four hours than that which takes place in other regions. The corresponding motor nerves will be, consequently, the first saturated by the toxine, and this saturation will be manifested by a localized contraction or by a local tetanus. However, in other regions the terminations of the nerves will absorb a certain quantity of toxine carried to them by the lymph. It is then that local tetanus will be succeeded by general tetanus. We are not yet able to decide whether the tetanus toxine can pass from the peripheral neuron to a sensory neuron with which it is connected, or if the contractions are to be attributed to the action of the toxine upon the one or the other of those cellular elements.

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[a] Marie, A., and Morax, V.: Recherches sur l'absorption de la toxine tetanique. Ann. Inst. Pasteur, vol. 18, 1902, p. 818–832.

In the absorption of the toxine it is necessary to consider not only the number of absorbing elements, but also the concentration of the solution of the toxine, for this concentration is always much more considerable at the point of inoculation than in other regions, where the toxine only diffuses in the mass of blood. This preponderating absorption by the nerve fibers at the inoculated region explains why the injection at several points of the body of a nonfatal dose of the toxine provokes the appearance of tetanus earlier and more severely than inoculation at a single point. When, instead of injecting the toxine under the skin or into the muscle, it is introduced directly into a viscus, the symptoms of tetanus appear only when the motor nerves are bathed in the toxine circulating in the blood.

In a later paper Marie and Morax[a] found that the central nerve substance of mammals, which possesses the power of neutralizing tetanus toxine, loses its power almost completely by drying in a vacuum. Some brain of a guinea pig finely bruised up is exposed under a vacuum where it loses about three-fourths of its weight. When they endeavored to repeat the experiments of Wassermann with this cerebral powder it was found that the nervous tissue had lost 97 per cent of its fixative power. This fixative power is not influenced by a dry heat of 60°, 100°, or 126° C. It is known, on the other hand, that the drying of antitetanic serum causes slight modification. The phenomenon of Wassermann would not seem, then, to be similar to the antitoxic action of specific sera.

The work of Meyer and Ransom[b] was done about the same time as the work referred to above by Morax and Marie, but not published till July, 1903. They found that the toxine is carried toward the centers by the motor nerves, and that this is the only way for the poison to reach the central nervous system. The toxine absorption by the nerve is dependent upon the integrity of the axis cylinder. A normal nerve retains the toxine for an hour and a half after intramuscular injection, a torn nerve about twenty-four hours; but if the nerve is degenerated it does not absorb the poison till about six hours later. If the nerve be cut before inoculation, there is no toxine in the proximal portion, but it is present in the distal portion. The toxine therefore enters the nerve through the muscle plates and not by the capillaries. After tearing a poisoned nerve the toxine disappears in a few hours in the proximal end, as the rupture stops the upper current. The passage of the toxine upward in the spinal cord is barred by section of the cord. The period of incubation is much shorter when the toxine is injected into the nerve than after subcutaneous or intravenous injections; injections into the cord have a still shorter period of incubation.

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[a] Morax, V., and Marie, A.: Note sur les propriétés fixatrices de la substance cérébrale desséchée. Bull de l'Inst. Pasteur, vol. 1, 1903, p. 41.

[b] Meyer, H., and Ransom, Fred.: Untersuchungen über den Tetanus. Arch. f. exper. Path. u. Pharm., vol. 49, 1903.

Their theory of tetanus is that the toxine is probably absorbed by the axis cylinder endings and reaches the corresponding spinal nerve center by that route; from the spinal center it spreads to the corresponding center in the other half of the cord and outwards, resulting in generalized tetanus. When intoxication is produced through the circulation, the poison is taken up by the nerve ends in all parts of the body and the disease is not localized, but general. The antitoxin, unlike the toxine, does not travel by the nerve route, but is found only in the blood and lymph.

Besredka[a] showed that the cerebral mass is capable of fixing more tetanus toxine than it is capable of neutralizing. The fixative substance of the brain is not then an antitoxic substance in the proper meaning of the word. The cerebral mass saturated with tetanus toxine recovers some of its primative integrity after the addition of true antitoxin, whether this comes from an animal of the same species or of a different species. The combination of the brain and toxine does not represent, then, a stability comparable to that which exists between the toxine and its true antitoxin. The phenomenon observed by Wassermann and Takaki ought then to be attributed to the presence in the cerebral matter of a particular substance other than the true tetanus antitoxin.

Dmitrievsky,[b] 1903, found that the brain of animals in which the immunization has been of only short duration and of which the blood does not possess high antitetanic power does not differ in its antitetanic property from a normal brain. The brain of animals which were immunized for a long time and of which the blood contained a large quantity of antitoxin is able to inactivate a much larger quantity of tetanus toxine than the brain of normal animals. This difference, however, is not considerable, for all the animals injected with the mixture of the poison and of brain from a guinea pig immunized presented some slight tetanic symptoms. The blood of animals of which the immunization has been continued a long time contains always more tetanus antitoxin than their brain.

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[a] Besredka, A.: De la fixation de la toxine tétanique par le cerveau. Ann. Inst. Pasteur, vol. 17, 1903, pp. 138–147.

[b] Dmitrievsky, K.: Recherches sur les propriétés antitétaniques des nerveaux de l'animal immunisé. Ann. Inst. Pasteur, vol. 17, 1903, pp. 148–160.

A second paper by Morax and Marie,[a] appeared in 1903, in which they showed that the affinity of tetanus toxine for the nerves seems to be shown *in vitro* by the experiments of Wassermann and is proved *in vivo* by the presence of this poison in the nerves of tetanized animals. It has been definitely established that it is the peripheral expansions of the neurons which absorb the toxine for transportation from point to point toward the nerve cell.

The object was to determine the respective neurons in this fixation and in this transportation of the toxine. The discovery of the poison in the nerve areas has shown that the three different types of nerve fibers constituting the cord, viz, the motor fibers, sensory, and sympathetic, are equally able to absorb the tetanus toxine. The considerable portion of this poison found in the massateric nerves is in conformity with the early appearance of tetanus in this region. The total absence of tetanus toxine in the optic nerves corroborates the anatomical findings which show them to be a prolongation of the brain, as it is known that the toxine is not found in the medulary neurons or brain, since it is fixed by them.

Blumenthal[b] concludes that:

> The cells of the body as well as the central nervous system have the property of binding tetanus toxine.
>
> The tetanus poison is altered in the organism as a consequence of this binding with the cells.
>
> There is an affinity of the antitoxin for the tetanus poison which has been bound in the tissues. This affinity is, however, comparatively slight in comparison to that it has for the toxine secreted by the cultures.

Tiberti,[c] 1905, concludes as follows:

> If we inject tetanus toxine subcutaneously into susceptible animals, the largest part first goes into the lymphatic vessels and from there into the blood. A smaller quantity is absorbed by the nerve endings and is carried by them to the nerve centers. After hypodermic injection of tetanus toxine in a limb the toxine-constant in the large nerves of that limb is demonstrable.
>
> The transportation of the tetanus toxine to the nerve centers by the nerve routes takes place not through the lymph channels, but in the plasma of the nerve fiber consisting of the axis cylinder. In order that the nerve fibers may be capable of taking up the tetanus toxine and conducting it to the nerve centers, it is necessary that the axis cylinder be normal.
>
> The tetanus toxine moves toward the nerve cells because the latter have a special affinity for tetanus toxine and in that way the nerve fibers become saturated.
>
> If tetanus toxine is injected into a muscle it diffuses throughout the muscle itself in the serous fluid. It is then taken up by the nerve endings in the muscle and by means of the nerves is carried to the center.

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[a] Morax, V., and Marie, A.: Recherches sur l'absorption de la toxine tétanique. Ann. Inst. Pasteur, vol. 17, no. 5. 1903. pp. 335–342.

[b] Blumenthal, Ferdinand.—Ueber das an die Organe gebundene Tetanusgift und seine Beziehung zum Antitoxin. Arch. f. physiol., 1904, pp. 217–220.

[c] Tiberti: Ueber den Transport des Tetanusgiftes zu den Rückenmarkszentren durch die Nervenfasern. Centblt. f. Bakt., 1 abt, vol. 38, 1905, pp. 281, 413, 499, 625.

Tetanus toxine inoculated into the gluteus muscle of a guinea pig is found in the corresponding ischiatic nerve in evident doses one and a half hours after the injection, whereas it is found much quicker in the blood, viz, after ten minutes.

Minimal doses of tetanus toxine are sufficient to cause severe symptoms of tetanus poisoning if injected into the parenchyma of a nerve. The same doses if injected under the skin or into the circulation produce no tetanic symptoms.

If tetanus antitoxin is injected into a nerve trunk and immediately thereafter tetanus toxine is inoculated into the corresponding muscle enervated by this nerve, it is possible to prevent the toxine reaching the corresponding nerve center and no symptoms of tetanus of any kind appear in the muscle area in question.

Tetanus toxine inoculated directly into the nerve has no other channel of transference to the nerve centers than the nerve substance itself. This fact is demonstrated by severing the spinal cord at a particular point, in which case the effect of the toxine is limited to the area corresponding to the section of the cord connected with the seat of the inoculation. And, further, the section of the spinal cord prevents the spreading of the toxine to the upper regions of the cord.

By cutting a particular nerve trunk the muscles innervated by this nerve show no indication of tetanus following the inoculation of tetanus toxine.

If tetanus antitoxin is injected directly into the substance of the spinal cord, then we see a decided abbreviation of the period of incubation, and such inoculation gives us a characteristic picture of the disease known as tetanus dolorosus.

If tetanus toxine is injected into the circulation, we have, after a longer or shorter period of incubation, dependent upon the animal species, all muscles simultaneously seized with tetanus contractures, because the tetanus toxine is absorbed by all the nerve trunks at the same time and thus conveyed to the nerve centers. In such cases we do not get the so-called local tetanus which is observed when the toxine is injected under the skin or into the parenchyma of the nerve. It requires a much stronger dose of toxine in order to produce symptoms of tetanus in an animal when the toxine is injected directly into the circulation than when it is given subcutaneously or directly into a nerve.

The tetanus toxine injected into the circulating blood quickly passes out into the lymph. The tetanus toxine can not be demonstrated with certainty in the cerebrospinal fluid.

Loew and Meyer[a] say that the characteristic symptoms of tetanus poisoning are to a certain extent due to the effects of the toxine upon the central nervous system. An exception was found by Dönitz in the condition called by him "tetanus sine tetano." He found that in intravenous injections in rabbits of tetanus toxine in quantities insufficient to produce local tetanus the animals nevertheless rapidly lost weight and died. Dönitz believed that the cause of cachexia was a parenchymatous degeneration. Loew and H. Meyer injected a number of rabbits with small quantities of tetanus toxine after the manner of Dönitz and found that some of them died without symptoms of tetanus and that some remained well. The animals that died showed symptoms of coccidial infection, and he therefore concluded that the cachexia was not caused by the tetanus toxine.

Loew and Meyer then conducted some experiments to determine whether animals could be rendered hypersusceptible to tetanus poison in the same sense that Behring and Kitashima showed in guinea pigs treated with small doses of diphtheria toxine. These investigations terminated negatively. The authors succeeded, however, in inducing hypersusceptibility to tetanus toxine in rabbits by the intradural injection of minute amounts.

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[a] Loew, O., and Meyer, H.: Zur Kenntniss der Tetanusvergiftung. Sitzungsb. d. Gesellsch. f. Beförd. d. ges. Naturw. zu Marb. (1904), 1905, p. 11–13.

From the above review it seems to have been definitely shown that the motor nerves have a specific affinity for tetanus toxine. When the toxine is given subcutaneously, the adjacent motor nerve-endings at once begin to take it up and transport it to the cord. The lymphatics also absorb much of it, and in a short while it appears in the blood stream, which carries it to all parts of the body, where it is absorbed by the motor nerve-endings, which are bathed in the toxine-laden fluid. The reason that the symptoms appear earlier in the limbs in which the toxine is injected is because the toxine-index, as it were, is higher there than in other parts of the body. When the toxine is given into the cord or into a nerve trunk, the incubation period is shortened; if into a nerve trunk, the tetanus is sharply localized at first, and in fact the animal may die before general tetanus sets in. When given into the circulation, generalized tetanus results in a short time. The weight of evidence is in favor of the view that the toxine produces its effect solely by the absorption of the toxine by the nerves, whose axis cylinder substance has a specific affinity for the poison. They transport it to the cord and brain through the axis cylinder substance itself.

THE GERMAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.[a]

The official testing of this serum is conducted at the Kgl. Institut für experimentelle Therapie zu Frankfurt a. M., of which Dr. Paul Ehrlich is the director. Tetanus antitoxine, like other curative sera, is examined in the Institut first for harmlessness and second for potency.

The serum is considered harmless if (1) it is wholly clear and free from much precipitate; (2) contains no bacterial contamination; (3) contains not more than 0.5 per cent phenol or 0.4 per cent trikresol; (4) is free from toxines, especially tetanus toxine.

The serum should not contain more than a very slight precipitate at the bottom and must under all circumstances be absolutely clear. The tests of the serum for sterility are made in accordance with the usual bacteriological methods. Five drops of the serum are planted into one agar plate, two glucose-bouillon test tubes, and a glucose-agar test tube with a sufficient quantity of the media for deep anærobic cultures. The inoculated plates and tubes must remain sterile. In order to test the quantity of phenol contained in the serum, a mouse weighing 15 grams is injected subcutaneously with 0.5 c. c. of the serum. If this mouse shows slight or no symptoms of poisoning, then the serum does not contain an excessive amount of the preservative allowed.

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[a] The account of the German methods of testing is taken from Otto's work entitled "Die Staatliche Prüfung der Heilsera," Jena, 1906. Also from Behring's "Die Wertbestimmung des Tetanus-antitoxins und seine Verwendung in der Menschenärztlichen und thierärztlichen Praxis," Deut. med. Woch., vol. 26, 1900, p. 29–52, etc.

A guinea pig is then injected subcutaneously with 10 c. c. of the serum in order to determine the presence of tetanus. If the guinea pig remains well, it is considered definite proof that the serum is free from this dangerous contamination. For this test it is necessary to use healthy, normal animals that have not been previously used for other purposes, so as to avoid the serum reaction.[a]

The official testing of antitoxin for potency at Ehrlich's institute is based entirely upon von Behring's method.[b] Von Behring proceeds as follows:

He dilutes the serum to be tested with physiological salt solution in the proportion of 1 c. c. of serum to 99 c. c. of the salt solution. One cubic centimeter of this dilution is then placed in an Erlenmeyer flask containing 38 c. c. of distilled water, and 1 c. c. of a high-grade test poison is added (the test poison No. $5 = \frac{N}{10}$). A uniform mixture of these three substances is obtained by shaking and the mixture is then allowed to stand thirty minutes, when a mouse of medium weight is injected subcutaneously with 0.4 c. c. If the mouse receiving 0.4 c. c. of this mixture remains well, then the serum contains at least 10 immunity units in 1 c. c., because 1 c. c. of the dilution 1:100 was entirely neutralized with 1 c. c. of the toxine, representing 0.1 normal poison. If the mouse after the subcutaneous inoculation of 0.4 c. c. of the mixture becomes tetanic, then the serum naturally contains somewhat less than 10 immunity units. If the mouse dies, we may form some judgment from the time intervening between the injection and death as to the approximate value of the serum.

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[a] Otto, R.: Das Theobald Smithsche Phänomen der Serum-Ueberempfindlichkeit. Leuthold-Gedenkschrift, Bd. 1.

Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the injection of horse serum. Bull. No. 29, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., 1906.

Rosenau, M. J., and Anderson, John F.: Studies upon hypersusceptibility and immunity. Bull. No. 36, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Wash., 1907.

[b] Behring and Knorr: Ueber den Immunisierungswert und Heilwert des Tetanusheilserums bei weissen Mäusen. Zeit. f. Hyg., vol. 13, 1893.

v. Behring: Allg. Therapie der Infektionskrankheiten. Berlin and Vienna, Urban & Schwarzenberg, 1899.

———: Die Wertbestimmung des Tetanusantitoxins und seine Verwendung in der menschenärztlichen und tierärztlichen Praxis. Deut. med. Woch., 1900, No. 2.

More precise determination of the potency of serums injected into mice which remain entirely well after the injection of 0.4 c. c. of the mixture is made by further experiments. If in this way we obtain the L° value of the serum, we are enabled by a simple process of calculation to determine the antitoxic worth of the serum. That quantity of serum in 0.4 c. c. of the mixture containing 0.01 c. c. of test poison No. 5 (=L° dose) contains exactly $\frac{1}{1000}$ of an immunity unit.

Behring's notations, generally used in the German literature, are as follows:

1+Ms represents the fatal dose for 1 gram of mouse.
1+M represents the fatal dose for 1 gram of guinea pig.
Ms^{15} represents 15 times the minimal lethal dose for 1 gram of mouse.
The toxine is always represented by the plus sign, the antitoxin by the minus sign.

The present modification of the methods for testing tetanus antitoxin at Ehrlich's institute are largely based upon the work of Marx,[a] who found that the tetanus toxine could be preserved in a stable condition in a dried state in Ehrlich vacuum tubes.

The official testing of antitoxin is carried out in the following manner: One of the vacuum tubes containing the poison and another containing the serum are carefully opened by means of a file, and a certain quantity of water, say 5 c. c., added to each tube from a graduated pipette. The tubes are then carefully closed with a burnt cork. Each tube—the one containing toxine and the other serum—is now shaken many times in order to insure the complete solution of its contents. The contents of each tube are next emptied and carefully washed out with the remaining quantity of the diluting fluid. The serum must be so diluted that 1 c. c. of the solution represents just 0.01 of an immunity unit. After this has been accomplished the mixture of the toxine and antitoxin *in vitro* is made. For this purpose two series of eight flasks each are necessary. One c. c. of the standard serum solution is placed in each flask of the first series; 1 c. c. of a dilution of the unknown serum to be tested is placed in each flask of the second series. To each series is now added the following test doses of toxine, varying in accordance with the toxine from 0.8 to 1.5 c. c. These doses are so calculated that all the values from L°

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[a] Marx, E.: Mitteilungen aus der prüfungstechnischen Praxis. Festschrift f. Robert Koch. Jena, Gustav Fischer, 1903.

———: Diagnostik, Serumtherapie und Prophylaxe. Bibliothek v. Coler, vol. 11, Berlin, 1902.

Dönitz, W.: Die Wertbemessung der Schutz- und Heilsera. Handbuch Kolle-Wassermann, vol. 4, part 2.

to L + will be found in the series. Water is now added to the toxine-antitoxin mixtures, so that each flask will contain a total of 4 c. c. These flasks are well corked and remain one-half hour at room temperature protected from daylight by means of lead shells. After this period white mice are inoculated. Corresponding to the mixtures made, two series of eight mice each are injected. Each mouse receives 0.4 c. c. of the particular mixture under the skin of the right hind foot. Each mouse, therefore, receives $\frac{1}{1000}$ of an immunity unit plus varying amounts of toxine. As the doses of the toxine are selected so that each series of eight mice will give all values from the L° to the L + limits, it is possible to determine from such a series all phases, as some animals will remain normal, others show symptoms of tetanus, and others die acutely.

If the unknown serum which is being tested has just the strength supposed for it in accordance with the above test, then the results in the two series of animals correspond precisely. If, however, it is weaker, there is a considerable difference in the results; from which it is easy to see about how much weaker the serum is than the standard serum. The following tables gives an example of tests made in accordance with the above explanation:

THE STATE TESTING OF THERAPEUTIC SERA.

Date: February 1. 1905.

Tetanus antitoxin: Manufacturer. X; No. y.

Sent in: January 31, 1905. Received: February 1, 1905.

Horses Nos. 4 and 6: Bled September 22 and 24. 1904. Quantity of serum sent in: 5 liters. Phenol content: 0.5 per cent. Strength claimed: 6 × normal.

Test for sterility: Sterile.

Test for phenol content: Mouse showed slight trembling.

Potency determination: 6 immunity units.

I. Standard serum: Dilution. 0.006 to 26 c. c.

II. Serum: X No. 11. Dilution. 1:600 (=1+19. 1+29, etc.).

III. Normal poison solution: To 1 c. c. of the diluted serum is added:

Poison.	Water.
0.8 c. c.	2.2 c. c.=4 c. c.
0.9 c. c.	2.1 c. c.=4 c.c.
1.0 c. c.	2.0 c. c.=4 c. c.
1.1 c. c.	1.9 c. c.=4 c. c.
1.2 c. c.	1.8 c. c.=4 c. c.
1.3 c. c.	1.7 c. c.=4 c. c.
1.4 c. c.	1.6 c. c.=4 c. c.
1.5 c. c.	1.5 c. c.=4 c. c.

The mixture of toxine and serum is allowed to stand half an hour at room temperature.

Quantity injected: 0.4 c. c.

Kind of animal: White mice (15 grams).

Kind of serum and date of test.	Result.	Kind of serum and date of test.	Result.
Standard serum.		*Serum No. y.*	
Mouse 1; poison, 0.8 c. c.; yellow head:		Mouse 9; poison, 0.8 c. c.; red head:	
February 2, 1905	0	February 2, 1905	0
February 3, 1905	0	February 3, 1905	0
February 4, 1905	0	February 4, 1905	0
February 5, 1905	0	February 5, 1905	0
February 6, 1905	0	February 6, 1905	0
February 7, 1905	0	February 7, 1905	0
Mouse 2; poison, 0.9 c. c.; yellow neck:		Mouse 10; poison, 0.9 c. c.; red neck:	
February 2, 1905	0	February 2, 1905	0
February 3, 1905	0	February 3, 1905	0
February 4, 1905	0(?)	February 4, 1905	0(?)
February 5, 1905	?	February 5, 1905	?
February 6, 1905	?	February 6, 1905	?
February 7, 1905	?	February 7, 1905	?
Mouse 3; poison, 1.0 c. c.; yellow back:		Mouse 11; poison, 1.0 c. c.; red back:	
February 2, 1905	?	February 2, 1905	?
February 3, 1905	Slight symptoms.	February 3, 1905	Slight symptoms.
February 4, 1905	Do.	February 4, 1905	Do.
February 5, 1905	Mild symptoms.	February 5, 1905	Mild symptoms.
February 6, 1905	Do.	February 6, 1905	Do.
February 7, 1905	Marked symptoms.	February 7, 1905	Marked symptoms.
Mouse 4; poison, 1.1 c. c.; yellow rump:		Mouse 12; poison, 1.1 c. c.; red rump:	
February 2, 1905	Slight symptoms.	February 2, 1905	Slight symptoms.
February 3, 1905	Mild symptoms.	February 3, 1905	Mild symptoms.
February 4, 1905	Marked symptoms.	February 4, 1905	Marked symptoms.
February 5, 1905	Do.	February 5, 1905	Do.
February 6, 1905	Do.	February 6, 1905	Do.
February 7, 1905	Very marked symptoms.	February 7, 1905	Very marked symptoms.
Mouse 5; poison, 1.2 c. c.; yellow right front foot:		Mouse 13; poison, 1.2 c. c.; red right front foot:	
February 2, 1905	Mild symptoms.	February 2, 1905	Mild symptoms.
February 3, 1905	Marked symptoms.	February 3, 1905	Marked symptoms.
February 4, 1905	Very marked symptoms.	February 4, 1905	Very marked symptoms.
February 5, 1905	Marked symptoms.	February 5, 1905	Marked symptoms.
February 6, 1905	Very severe symptoms.	February 6, 1905	Very severe symptoms.
February 7, 1905	Severe symptoms.	February 7, 1905	Severe symptoms.
Mouse 6; poison, 1.3 c. c.; yellow left front foot:		Mouse 14; poison, 1.3 c. c.; red left front foot:	
February 2, 1905	Mild symptoms.	February 2, 1905	Mild symptoms.
February 3, 1905	Very marked symptoms.	February 3, 1905	Very marked symptoms.
February 4, 1905	Marked symptoms.	February 4, 1905	Marked symptoms.
February 5, 1905	Very severe symptoms.	February 5, 1905	Very severe symptoms.
February 6, 1905	Severe symptoms.	February 6, 1905	Severe symptoms.
February 7, 1905	Violent tetanus.	February 7, 1905	Violent tetanus.

Kind of serum and date of test.	Result.	Kind of serum and date of test.	Result.
Standard serum—Cont'd.		*Serum No. y*—Cont'd.	
Mouse 7; poison, 1.4 c. c.; yellow right hind foot:		Mouse 15; poison, 1.4 c. c.; red right hind foot:	
February 2, 1905......	Marked symptoms.	February 2, 1905......	Marked symptoms.
February 3, 1905......	Very severe symptoms.	February 3, 1905......	Very severe symptoms.
February 4, 1905......	Tetany.	February 4, 1905......	Tetany.
February 5, 1905......	Dead, 4.	February 5, 1905......	Dead, 4.
Mouse 8; poison, 1.5 c. c.; yellow left hind foot:		Mouse 16; poison, 1.5 c. c.; red left hind foot:	
February 2, 1905......	Very severe symptoms.	February 2, 1905......	Very severe symptoms.
February 3, 1905......	Tetanus.	February 3, 1905......	Tetanus.
February 4, 1905......	Dead, 3.	February 4, 1905......	Dead, 3.

In the above illustrated case the two series of animals reacted precisely the same. It therefore shows that the antitoxin tested was in reality six times the normal strength.

It is evident, however, that the standardization of tetanus antitoxin by the German method is much more complicated than the testing of diphtheria antitoxin.[a]

The following preparations of tetanus antitoxin are found upon the German market (1904):

1. V. Behring's tetanus curative serum, made by Professor v. Behring, officially tested by Professor Ehrlich at the Frankfurt Institut for experimentelle Therapie. It is sold by the Marbourg firm of Doctor Siebert and Doctor Ziegenbein. The preparation is sold in two amounts, the one containing 100 immunity units (the curative dose) and the other 20 immunity units (for prophylactic use). Small vials of dried antitoxin, containing 20 immunity units, for dusting wounds, are also sold.

2. Tizzoni's tetanus serum, made by Tizzoni and Cattani. This is sold by the firm of Merck, in Darmstadt. The preparation is sold in original vials of 5 grams each (normal dose) = 5,000,000 immunity units.

THE ITALIAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.

The Tizzoni preparation is now sold by Merck, at Darmstadt, and is said to contain in every cubic centimeter 80,000 immunity units (I. U.); in other words, the antitoxin amount which will neutralize 80,000 toxine units. The toxine unit, according to this reckoning, is that amount of toxine which will kill a rabbit weighing 1 kilogram in from four to five days. The dried antitoxin is usually furnished, 0.1 of a gram of which represents 1 c. c. of serum.

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[a] V. Lingelsheim: Immunität bei Tetanus. Kolle & Wassermann's Handbuch, vol. 4, part 2.

The Italian method is based entirely upon the work and theoretic considerations of Prof. Guido Tizzoni[a] and Giuseppina Cattani.

Tizzoni early abandoned the methods of standardizing tetanus antitoxin by injecting the serum and the poison separately into the animal with an interval of twenty-four hours. He soon found that the method of Ehrlich, Kossel, and Wassermann, which consists of injections of mixtures *in vitro* of toxine and antitoxin (of diphtheria), gives more accurate and reliable results. In applying this method to tetanus, Tizzoni encountered certain difficulties, due more particularly to the unstable nature of the tetanus toxine. He correctly observes that "Nothing more embarrassing can be imagined than experimenting with a toxine which changes in the hands of the experimenter and the precise grade of the potency of which can not be known at a given moment. Researches pursued under these conditions are long, laborious, often contradictory, uncertain, and confused; the conclusions derived from them are rather deductions than demonstrations; it is impossible to institute a continuous series of experiments, and the few certain results obtained are arrived at almost by accident in the midst of a farrago of observations which are of no service on account of the defects, in greater or less degree, in the dose of toxine used." A stable toxine from tetanus cultures can not be obtained. Cultures reach their maximum toxicity from the seventh to the fifteenth day and then gradually weaken, even when kept cold and protected from light. Filtration weakens the poison. Tizzoni found that filtrates even when kept under oil, in vacuo, or under gases lose their toxic value.

Tizzoni and Kitasato independently found that if filtered cultures are reduced to dryness in vacuo at a low temperature (20°–22° C.) the resulting yellow-brown scales gradually lose toxicity. No better success was had with Brieger and Frankel's method of precipitation with alcohol. It seems that Tizzoni was the first to obtain a precipitated tetanus toxine with ammonium sulphate, following the method of Brieger and Frankel with other toxines. Buchner and Rapp used the same method and obtained a stable poison. But Tizzoni found these precipitates not to be constant. He believes that these precipitates first decompose rapidly and then very slowly, so that they contain a large quantity of transformed toxine, capable of confusing the results of experiments.

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[a] The information in regard to the Italian method of standardizing tetanus antitoxin was gotten from "Sul Modo di Determinare la Potenza del Sero Antitetanico, col metodo della mescolanza in vitro" by Prof. Guido Tizzoni, in "Memorie della r. Accademia delle Scienze dell' Instituto di Bologna." Tomo 8. Serie Quinta. Bologna, 1899–1900. Professor Tizzoni's entire article was kindly translated for us by Miss E. R. Jones.

Tizzoni believes that the weakening of the dried precipitated toxin is due to the production of an acid substance. It was for this reason that Knorr dissolved the precipitate in a 10 per cent solution of sodium chloride.

Tizzoni at one time believed the instability of the precipitated toxine to be due to the ammonium sulphate which remained in the precipitate and eliminated this by spreading the fresh moist precipitate upon porous porcelain; but the toxine so obtained showed itself to be no more stable than those that preceded it. On the other hand, he obtained very satisfactory results by the elimination of the sulphate of ammonium by means of dialysis. Tizzoni claims that tetanus toxine does not dialyze. He recognizes that the stability of the toxine so prepared is not indefinite, for he states that this dialyzed precipitate passes through some modification in the course of time. The toxine, however, is sufficiently stable for the purpose of standardizing antitoxin and for scientific tests. Besides serving for the preparation of the standard antitoxin, this method is applicable for concentrating the poison for injection into animals. Tizzoni showed further that almost all of the toxines produced by a culture are precipitated by ammonium sulphate—that is, there is very little loss in this process.

Tizzoni found that antitoxic serums measured against fresh tetanus toxine and an old and weakened poison gave different results. He believes that this is due largely to the modified toxine or "toxoid" contained in the weakened serum.

Tizzoni considers it of importance to use for the preparation of the toxine, cultures which at the period of their greatest development contain the greatest degree of toxicity. Thus 0.001 c. c. of the filtrate should constantly kill 1 kilogram of rabbit in four days. Cultures which at the moment of precipitation with sulphate of ammonium do not possess this toxicity are rejected.

In view of the differences shown by different species of animals Tizzoni always uses the rabbit for standardizing both toxine and antitoxin, and finds it necessary to take into account the weight of the animal. He concluded that the principal condition to be realized in the standardizing of tetanus antitoxin is the possession of a stable toxine. He dwells upon the importance of determining the therapeutic value of the serums in relation to the antitoxic value as determined by animal tests. Too much confusion prevails to-day in the method of representing the value of tetanus antitoxic serums. This condition should be terminated by adopting a language common to all and easily understood. In the present state of affairs the confusion has arrived at such a point that anyone concerned in a discussion can not judge from the value attributed to two serums of different origin the real relation of their potencies. Tizzoni therefore pleads for a simplification and unification of methods, and commends his own for general use. Tizzoni prepares two solutions—one of toxine and one of serum—the first in distilled water and the second in 0.75 per cent salt solution. The two solutions are mixed, and after one-half hour of contact the entire mixture is injected into the posterior part of the thigh of a rabbit weighing about 1 kilogram. The value of the serum is always calculated upon the titre of the mixture which does not cause symptoms in an animal.

THE FRENCH METHOD OF STANDARDIZING TETANUS ANTITOXIN.

The strength of the tetanus antitoxin manufactured by the Pasteur Institute is not stated upon the packages sold by the Pasteur Vaccine Company. The prophylactic dose is given as 10 c. c. It is stated that the antitoxic dose of the serum amounts to 1,000,000,000; that is, to protect a mouse against the smallest fatal dose it is sufficient to use $\frac{1}{1,000,000,000}$ of its weight of serum.

The description of the method used in France was kindly sent to us in a personal communication by Professor Arloing, of the Bacteriological Institute of Lyons, having been prepared for him by Professor Courmont, who has charge of the control of antitetanic serum in that institute.

A number of guinea pigs are injected under the skin with quantities of serum equal to $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, $\frac{1}{10,000,000}$, $\frac{1}{10,000,000}$, and even $\frac{1}{1,000,000,000}$ of their weight. Twelve to twenty hours afterwards they, as well as the controls, are inoculated subcutaneously with a single fatal dose of tetanus toxine. The time of death of those which develop tetanus is noted. The serum is then said to have an immunizing or antitoxic power of $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, etc., according as the guinea pig which received $\frac{1}{500,000}$, $\frac{1}{1,000,000}$, etc., of its weight of serum has survived the injection of tetanus toxine fatal for the control.

Professor Courmont adds that their serum is usually of a strength of $\frac{1}{1,000,000}$ and never goes below $\frac{1}{500,000}$.

The French antitoxic serums are made at the Institut Pasteur, Paris, and l'Institut Bacteriologique, Lyon.

TABLES FOR DILUTING THE TOXINE AND ANTITOXIN.

The following tables are used for diluting the tetanus toxines and antitoxins in this laboratory. They are published not only because they will save time and the possibility of error incident to such calculations, but because it is of some importance that the same methods of dilution be used by different laboratories in order that the results may be strictly comparable. This apparently trifling matter is of some importance in tetanus work on account of the known influence of concentration upon the tetanus poison and its antibody.

TABLE No. 8.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1000 1 c. c.=0.001

0.7 c. c. of B=0.0007 or 1/1, 430
.8 c. c. of B= .0008 or 1/1, 250
.9 c. c. of B= .0009 or 1/1, 111
1.0 c. c. of B= .0010 or 1/1, 000
1.1 c. c. of B= .0011 or 1/ 909
1.2 c. c. of B= .0012 or 1/ 833
1.3 c. c. of B= .0013 or 1/ 769
1.4 c. c. of B= .0014 or 1/ 714
1.5 c. c. of B= .0015 or 1/ 666
1.6 c. c. of B= .0016 or 1/ 625
1.7 c. c. of B= .0017 or 1/ 588
1.8 c. c. of B= .0018 or 1/ 555
1.9 c. c. of B= .0019 or 1/ 526
2.0 c. c. of B= .0020 or 1/ 500
2.1 c. c. of B= .0021 or 1/ 476
2.2 c. c. of B=0.0022 or 1/454
2.3 c. c. of B= .0023 or 1/435
2.4 c. c. of B= .0024 or 1/417
2.5 c. c. of B= .0025 or 1/400
2.6 c. c. of B= .0026 or 1/384
2.7 c. c. of B= .0027 or 1/370
2.8 c. c. of B= .0028 or 1/357
2.9 c. c. of B= .0029 or 1/345
3.0 c. c. of B= .0030 or 1/333
3.1 c. c. of B= .0031 or 1/322
3.2 c. c. of B= .0032 or 1/312
3.3 c. c. of B= .0033 or 1/303
3.4 c. c. of B= .0034 or 1/294
3.5 c. c. of B= .0035 or 1/285

TABLE No. 9.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+19=1:2000 1 c. c.=0.0005

0.7 c. c. of B=0.00035 or 1/2, 860
.8 c. c. of B= .00040 or 1/2, 500
.9 c. c. of B= .00045 or 1/2, 222
1.0 c. c. of B= .00050 or 1/2, 000
1.1 c. c. of B= .00055 or 1/1, 818
1.2 c. c. of B= .00060 or 1/1, 666
1.3 c. c. of B= .00065 or 1/1, 538
1.4 c. c. of B= .00070 or 1/1, 428
1.5 c. c. of B= .00075 or 1/1, 333
1.6 c. c. of B= .00080 or 1/1, 250
1.7 c. c. of B= .00085 or 1/1, 174
1.8 c. c. of B= .00090 or 1/1, 111
1.9 c. c. of B= .00095 or 1/1, 057
2.0 c. c. of B= .00100 or 1/1, 000
2.1 c. c. of B= .00105 or 1/ 952
2.2 c. c. of B=0.00110 or 1/909
2.3 c. c. of B= .00115 or 1/869
2.4 c. c. of B= .00120 or 1/833
2.5 c. c. of B= .00125 or 1/800
2.6 c. c. of B= .00130 or 1/769
2.7 c. c. of B= .00135 or 1/741
2.8 c. c. of B= .00140 or 1/714
2.9 c. c. of B= .00145 or 1/689
3.0 c. c. of B= .00150 or 1/666
3.1 c. c. of B= .00155 or 1/645
3.2 c. c. of B= .00160 or 1/625
3.3 c. c. of B= .00165 or 1/606
3.4 c. c. of B= .00170 or 1/588
3.5 c. c. of B= .00175 or 1/571

Table No. 10.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+29=1:3000 1 c. c.=0.00033

0.7 c. c. of B=0.00024 or 1/4,170
.8 c. c. of B= .00027 or 1/3,700
.9 c. c. of B= .00030 or 1/3,333
1.0 c. c. of B= .000333333 or 1/3,000
1.1 c. c. of B= .00036 or 1/2,777
1.2 c. c. of B= .00040 or 1/2,500
1.3 c. c. of B= .00043 or 1/2,302
1.4 c. c. of B= .00046 or 1/2,173
1.5 c. c. of B= .00050 or 1/2,000
1.6 c. c. of B= .00053 or 1/1,850
1.7 c. c. of B= .00056 or 1/1,786
1.8 c. c. of B= .00060 or 1/1,666
1.9 c. c. of B= .00063 or 1/1,587
2.0 c. c. of B= .00066 or 1/1,515
2.1 c. c. of B= .00070 or 1/1,428

2.2 c. c. of B=0.00073 or 1/1,369
2.3 c. c. of B= .00076 or 1/1,304
2.4 c. c. of B= .00080 or 1/1,250
2.5 c. c. of B= .00083 or 1/1,200
2.6 c. c. of B= .00086 or 1/1,154
2.7 c. c. of B= .00090 or 1/1,111
2.8 c. c. of B= .00093 or 1/1,071
2.9 c. c. of B= .00096 or 1/1,034
3.0 c. c. of B= .00100 or 1/1,000
3.1 c. c. of B= .00103 or 1/ 967
3.2 c. c. of B= .00107 or 1/ 937
3.3 c. c. of B= .00110 or 1/ 909
3.4 c. c. of B= .00113 or 1/ 882
3.5 c. c. of B= .00116 or 1/ 857

Table No. 11.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+39=1:4000 1 c. c.=0.00025

0.7 c. c. of B=0.000175 or 1/5,714
.8 c. c. of B= .000200 or 1/5,000
.9 c. c. of B= .000225 or 1/4,444
1.0 c. c. of B= .000250 or 1/4,000
1.1 c. c. of B= .000275 or 1/3,636
1.2 c. c. of B= .000300 or 1/3,333
1.3 c. c. of B= .000325 or 1/3,077
1.4 c. c. of B= .000350 or 1/2,860
1.5 c. c. of B= .000375 or 1/2,666
1.6 c. c. of B= .000400 or 1/2,500
1.7 c. c. of B= .000425 or 1/2,353
1.8 c. c. of B= .000450 or 1/2,222
1.9 c. c. of B= .000475 or 1/2,105
2.0 c. c. of B= .000500 or 1/2,000
2.1 c. c. of B= .000525 or 1/1,905

2.2 c. c. of B=0.000550 or 1/1,818
2.3 c. c. of B= .000575 or 1/1,739
2.4 c. c. of B= .000600 or 1/1,666
2.5 c. c. of B= .000625 or 1/1,600
2.6 c. c. of B= .000650 or 1/1,538
2.7 c. c. of B= .000675 or 1/1,481
2.8 c. c. of B= .000700 or 1/1,428
2.9 c. c. of B= .000725 or 1/1,379
3.0 c. c. of B= .000750 or 1/1,333
3.1 c. c. of B= .000775 or 1/1,290
3.2 c. c. of B= .000800 or 1/1,250
3.3 c. c. of B= .000825 or 1/1,212
3.4 c. c. of B= .000850 or 1/1,174
3.5 c. c. of B= .000875 or 1/1,143

TABLE NO. 12.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A) +49=1:5000 1 c. c.=0.0002

0.7 c. c. of B=0.00014 or 1/7,143
.8 c. c. of B= .00016 or 1/6,250
.9 c. c. of B= .00018 or 1/5,555
1.0 c. c. of B= .00020 or 1/5,000
1.1 c. c. of B= .00022 or 1/4,545
1.2 c. c. of B= .00024 or 1/4,170
1.3 c. c. of B= .00026 or 1/3,846
1.4 c. c. of B= .00028 or 1/3,571
1.5 c. c. of B= .00030 or 1/3,333
1.6 c. c. of B= .00032 or 1/3,125
1.7 c. c. of B= .00034 or 1/2,941
1.8 c. c. of B= .00036 or 1/2,777
1.9 c. c. of B= .00038 or 1/2,631
2.0 c. c. of B= .00040 or 1/2,500
2.1 c. c. of B= .00042 or 1/2,381
2.2 c. c. of B=0.00044 or 1/2,272
2.3 c. c. of B= .00046 or 1/2,173
2.4 c. c. of B= .00048 or 1/2,083
2.5 c. c. of B= .00050 or 1/2,000
2.6 c. c. of B= .00052 or 1/1,923
2.7 c. c. of B= .00054 or 1/1,851
2.8 c. c. of B= .00056 or 1/1,786
2.9 c. c. of B= .00058 or 1/1,724
3.0 c. c. of B= .00060 or 1/1,666
3.1 c. c. of B= .00062 or 1/1,613
3.2 c. c. of B= .00064 or 1/1,562
3.3 c. c. of B= .00066 or 1/1,515
3.4 c. c. of B= .00068 or 1/1,470
3.5 c. c. of B= .00070 or 1/1,428

TABLE NO. 13.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A) +59=1:6000 1 c.c.=0.00017

0.7 c. c. of B=0.000117 or 1/8,571
.8 c. c. of B= .000133 or 1/7,500
.9 c. c. of B= .000150 or 1/6,666
1.0 c. c. of B= .000166666 or 1/6,000
1.1 c. c. of B= .000183 or 1/5,454
1.2 c. c. of B= .000199 or 1/5,000
1.3 c. c. of B= .000216 or 1/4,615
1.4 c. c. of B= .000233 or 1/4,285
1.5 c. c. of B= .000249 or 1/4,000
1.6 c. c. of B= .000266 or 1/3,750
1.7 c. c. of B= .000283 or 1/3,529
1.8 c. c. of B= .000300 or 1/3,333
1.9 c. c. of B= .000317 or 1/3,158
2.0 c. c. of B= .000333 or 1/3,000
2.1 c. c. of B= .000350 or 1/2,857
2.2 c. c. of B=0.000367 or 1/2,727
2.3 c. c. of B= .000383 or 1/2,609
2.4 c. c. of B= .000400 or 1/2,500
2.5 c. c. of B= .000417 or 1/2,400
2.6 c. c. of B= .000433 or 1/2,307
2.7 c. c. of B= .00045 or 1/2,222
2.8 c. c. of B= .000467 or 1/2,143
2.9 c. c. of B= .000483 or 1/2,069
3.0 c. c. of B= .000500 or 1/2,000
3.1 c. c. of B= .000517 or 1/1,938
3.2 c. c. of B= .000533 or 1/1,875
3.3 c. c. of B= .000550 or 1/1,818
3.4 c. c. of B= .000567 or 1/1,764
3.5 c. c. of B= .000583 or 1/1,714

TABLE No. 14.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+69=1:7000 1 c. c.=0.000142857

0.7 c. c. of B=	0.000100	or 1/10, 000	2.2 c. c. of B=	0.000314	or 1/3, 181
.8 c. c. of B=	.000114	or 1/8, 750	2.3 c. c. of B=	.000329	or 1/3, 043
.9 c. c. of B=	.000129	or 1/7, 777	2.4 c. c. of B=	.000343	or 1/2, 916
1.0 c. c. of B=	.000142857	or 1/7, 000	2.5 c. c. of B=	.000357	or 1/2, 800
1.1 c. c. of B=	.000157	or 1/6, 363	2.6 c. c. of B=	.000371	or 1/2, 692
1.2 c. c. of B=	.000171	or 1/5, 833	2.7 c. c. of B=	.000385	or 1/2, 592
1.3 c. c. of B=	.000185	or 1/5, 385	2.8 c. c. of B=	.000400	or 1/2, 500
1.4 c. c. of B=	.000200	or 1/5, 000	2.9 c. c. of B=	.000414	or 1/2, 414
1.5 c. c. of B=	.000214	or 1/4, 666	3.0 c. c. of B=	.000429	or 1/2, 333
1.6 c. c. of B=	.000229	or 1/4, 375	3.1 c. c. of B=	.000443	or 1/2, 258
1.7 c. c. of B=	.000243	or 1/4, 118	3.2 c. c. of B=	.000457	or 1/2, 187
1.8 c. c. of B=	.000257	or 1/3, 888	3.3 c. c. of B=	.000471	or 1/2, 121
1.9 c. c. of B=	.000271	or 1/3, 684	3.4 c. c. of B=	.000485	or 1/2, 059
2.0 c. c. of B=	.000285	or 1/3, 500	3.5 c. c. of B=	.000500	or 1/2, 000
2.1 c. c. of B=	.000300	or 1/3, 333			

TABLE No. 15.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+79=1:8000 1 c. c.=0.000125

0.7 c. c. of B=	0.0000875	or 1/11, 428	2.2 c. c. of B=	0.0002750	or 1/3, 636
.8 c. c. of B=	.0001000	or 1/10, 000	2.3 c. c. of B=	.0002875	or 1/3, 478
.9 c. c. of B=	.0001125	or 1/8, 888	2.4 c. c. of B=	.0003000	or 1/3, 333
1.0 c. c. of B=	.0001250	or 1/8, 000	2.5 c. c. of B=	.0003125	or 1/3, 200
1.1 c. c. of B=	.0001375	or 1/7, 272	2.6 c. c. of B=	.0003250	or 1/3, 077
1.2 c. c. of B=	.0001500	or 1/6, 666	2.7 c. c. of B=	.0003375	or 1/2, 962
1.3 c. c. of B=	.0001625	or 1/6, 154	2.8 c. c. of B=	.0003500	or 1/2, 860
1.4 c. c. of B=	.0001750	or 1/5, 714	2.9 c. c. of B=	.0003625	or 1/2, 758
1.5 c. c. of B=	.0001875	or 1/5, 333	3.0 c. c. of B=	.0003750	or 1/2, 666
1.6 c. c. of B=	.0002000	or 1/5, 000	3.1 c. c. of B=	.0003875	or 1/2, 581
1.7 c. c. of B=	.0002125	or 1/4, 706	3.2 c. c. of B=	.0004000	or 1/2, 500
1.8 c. c. of B=	.0002250	or 1/4, 444	3.3 c. c. of B=	.0004125	or 1/2, 424
1.9 c. c. of B=	.0002375	or 1/4, 210	3.4 c. c. of B=	.0004250	or 1/2, 353
2.0 c. c. of B=	.0002500	or 1/4, 000	3.5 c. c. of B=	.0004375	or 1/2, 286
2.1 c. c. of B=	.0002625	or 1/3, 809			

Table No. 16.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+89=1:9,000 1 c. c.=0.000111

0.7 c. c. of B=0.000077 or 1/12,859
.8 c. c. of B= .000088 or 1/11,250
.9 c. c. of B= .000100 or 1/10,000
1.0 c. c. of B= .000111111 or 1/9,000
1.1 c. c. of B= .000122 or 1/8,173
1.2 c. c. of B= .000133 or 1/7,500
1.3 c. c. of B= .000144 or 1/6,923
1.4 c. c. of B= .000155 or 1/6,428
1.5 c. c. of B= .000166 or 1/6,000
1.6 c. c. of B= .000177 or 1/5,625
1.7 c. c. of B= .000188 or 1/5,293
1.8 c. c. of B= .000200 or 1/5,000
1.9 c. c. of B= .000211 or 1/4,736
2.0 c. c. of B= .000222 or 1/4,500
2.1 c. c. of B= .000233 or 1/4,286
2.2 c. c. of B=0.000244 or 1/4,091
2.3 c. c. of B= .000255 or 1/3,913
2.4 c. c. of B= .000266 or 1/3,750
2.5 c. c. of B= .000277 or 1/3,600
2.6 c. c. of B= .000288 or 1/3,461
2.7 c. c. of B= .000300 or 1/3,333
2.8 c. c. of B= .000311 or 1/3,214
2.9 c. c. of B= .000322 or 1/3,103
3.0 c. c. of B= .000333 or 1/3,000
3.1 c. c. of B= .000344 or 1/2,903
3.2 c. c. of B= .000355 or 1/2,812
3.3 c. c. of B= .000366 or 1/2,727
3.4 c. c. of B= .000377 or 1/2,647
3.5 c. c. of B= .000388 or 1/2,571

Table No. 17.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+99=1:10,000 1 c. c.=0.0001

0.7 c. c. of B=0.00007 or 1/14,285
.8 c. c. of B= .00008 or 1/12,500
.9 c. c. of B= .00009 or 1/11,111
1.0 c. c. of B= .00010 or 1/10,000
1.1 c. c. of B= .00011 or 1/9,090
1.2 c. c. of B= .00012 or 1/8,333
1.3 c. c. of B= .00013 or 1/7,692
1.4 c. c. of B= .00014 or 1/7,143
1.5 c. c. of B= .00015 or 1/6,666
1.6 c. c. of B= .00016 or 1/6,250
1.7 c. c. of B= .00017 or 1/5,882
1.8 c. c. of B= .00018 or 1/5,555
1.9 c. c. of B=0.00019 or 1/5,263
2.0 c. c. of B= .00020 or 1/5,000
2.1 c. c. of B= .00021 or 1/4,762
2.2 c. c. of B= .00022 or 1/4,545
2.3 c. c. of B= .00023 or 1/4,348
2.4 c. c. of B= .00024 or 1/4,166
2.5 c. c. of B= .00025 or 1/4,000
2.6 c. c. of B= .00026 or 1/3,846
2.7 c. c. of B= .00027 or 1/3,703
2.8 c. c. of B= .00028 or 1/3,571
2.9 c. c. of B= .00029 or 1/3,448
3.0 c. c. of B= .00030 or 1/3,333

Table No. 18.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+19=1:20,000 1 c. c.=0.00005

0.7 c. c. of C=0.000035 or 1/28,571
.8 c. c. of C= .000040 or 1/25,000
.9 c. c. of C= .000045 or 1/22,222
1.0 c. c. of C= .000050 or 1/20,000
1.1 c. c. of C= .000055 or 1/18,181
1.2 c. c. of C= .000060 or 1/16,666
1.3 c. c. of C= .000065 or 1/15,385
1.4 c. c. of C= .000070 or 1/14,285
1.5 c. c. of C= .000075 or 1/13,333
1.6 c. c. of C= .000080 or 1/12,500
1.7 c. c. of C= .000085 or 1/11,764
1.8 c. c. of C= .000090 or 1/11,111
1.9 c. c. of C=0.000095 or 1/10,526
2.0 c. c. of C= .000100 or 1/10,000
2.1 c. c. of C= .000105 or 1/9,524
2.2 c. c. of C= .000110 or 1/9,090
2.3 c. c. of C= .000115 or 1/8,695
2.4 c. c. of C= .000120 or 1/8,333
2.5 c. c. of C= .000125 or 1/8,000
2.6 c. c. of C= .000130 or 1/7,615
2.7 c. c. of C= .000135 or 1/7,407
2.8 c. c. of C= .000140 or 1/7,143
2.9 c. c. of C= .000145 or 1/6,896
3.0 c. c. of C= .000150 or 1/6,666

Table No. 19.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+29=1:30,000 1 c. c.=0.0000333

0.7 c. c. of C=0.0000233 or 1/42,857
.8 c. c. of C= .0000266 or 1/37,500
.9 c. c. of C= .00003 or 1/33,333
1.0 c. c. of C= .0000333 or 1/30,000
1.1 c. c. of C= .0000366 or 1/37,322
1.2 c. c. of C= .00004 or 1/25,000
1.3 c. c. of C= .0000433 or 1/23,095
1.4 c. c. of C= .0000466 or 1/21,428
1.5 c. c. of C= .00005 or 1/20,000
1.6 c. c. of C= .0000533 or 1/18,761
1.7 c. c. of C= .0000566 or 1/17,668
1.8 c. c. of C= .00006 or 1/16,666
1.9 c. c. of C=0.0000633 or 1/15,799
2.0 c. c. of C= .0000666 or 1/15,000
2.1 c. c. of C= .00007 or 1/14,285
2.2 c. c. of C= .0000733 or 1/13,642
2.3 c. c. of C= .0000766 or 1/13,055
2.4 c. c. of C= .00008 or 1/12,500
2.5 c. c. of C= .0000833 or 1/12,000
2.6 c. c. of C= .0000866 or 1/11,547
2.7 c. c. of C= .00009 or 1/11,111
2.8 c. c. of C= .0000933 or 1/10,504
2.9 c. c. of C= .0000966 or 1/10,352
3.0 c. c. of C= .0001 or 1/10,000

Table No. 20.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+39=1:40,000 1 c. c.=0.000025

0.7 c. c. of C=0.0000175 or 1/57,142
.8 c. c. of C= .00002 or 1/50,000
.9 c. c. of C= .0000225 or 1/44,444
1.0 c. c. of C= .000025 or 1/40,000
1.1 c. c. of C= .0000275 or 1/36,363
1.2 c. c. of C= .00003 or 1/33,333
1.3 c. c. of C= .0000325 or 1/30,769
1.4 c. c. of C= .000035 or 1/28,571
1.5 c. c. of C= .0000375 or 1/26,666
1.6 c. c. of C= .00004 or 1/25,000
1.7 c. c. of C= .0000425 or 1/23,529
1.8 c. c. of C= .000045 or 1/22,222
1.9 c. c. of C=0.0000475 or 1/21,053
2.0 c. c. of C= .00005 or 1/20,000
2.1 c. c. of C= .0000525 or 1/19,048
2.2 c. c. of C= .000055 or 1/18,181
2.3 c. c. of C= .0000575 or 1/17,391
2.4 c. c. of C= .00006 or 1/16,666
2.5 c. c. of C= .0000625 or 1/16,000
2.6 c. c. of C= .000065 or 1/15,384
2.7 c. c. of C= .0000675 or 1/14,814
2.8 c. c. of C= .00007 or 1/14,285
2.9 c. c. of C= .0000725 or 1/13,793
3.0 c. c. of C= .000075 or 1/13,333

Table No. 21.

A.— 1+99=1:100 1 c. c.=0.01
B.—(1 of A)+ 9=1:1,000 1 c. c.=0.001
C.—(1 of B)+49=1:50,000 1 c. c.=0.00002

0.7 c. c. of C=0.000014 or 1/71,428
.8 c. c. of C= .000016 or 1/62,500
.9 c. c. of C= .000018 or 1/55,555
1.0 c. c. of C= .000020 or 1/50,000
1.1 c. c. of C= .000022 or 1/45,454
1.2 c. c. of C= .000024 or 1/41,666
1.3 c. c. of C= .000026 or 1/38,431
1.4 c. c. of C= .000028 or 1/35,714
1.5 c. c. of C= .000030 or 1/33,333
1.6 c. c. of C= .000032 or 1/31,250
1.7 c. c. of C= .000034 or 1/29,412
1.8 c. c. of C= .000036 or 1/27,777
1.9 c. c. of C=0.000038 or 1/26,316
2.0 c. c. of C= .000040 or 1/25,000
2.1 c. c. of C= .000042 or 1/23,809
2.2 c. c. of C= .000044 or 1/22,818
2.3 c. c. of C= .000046 or 1/21,739
2.4 c. c. of C= .000048 or 1/20,833
2.5 c. c. of C= .000050 or 1/20,000
2.6 c. c. of C= .000052 or 1/19,230
2.7 c. c. of C= .000054 or 1/18,518
2.8 c. c. of C= .000056 or 1/17,857
2.9 c. c. of C= .000058 or 1/17,241
3.0 c. c. of C= .000060 or 1/16,666

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis*; by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horse-hair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous zoological papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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O

TREASURY DEPARTMENT.
Public Health and Marine-Hospital Service of the United States.

HYGIENIC LABORATORY.—BULLETIN No. 44.
MAY, 1908.

REPORT No. 2

ON

THE ORIGIN AND PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA.

(1907.)

BY

M. J. ROSENAU, L. L. LUMSDEN,
and JOSEPH H. KASTLE.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1908.

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United States Public Health and Marine-Hospital Service.

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INTRODUCTION

This bulletin gives the results of our studies on typhoid fever in the District of Columbia during 1907. Our first report on this subject, for the year 1906, is contained in Hygienic Laboratory Bulletin No. 35.

This year our studies included epidemiological investigations of the disease during the typhoid fever season, daily bacteriological examinations of the raw and filtered Potomac River water, bacteriological and chemical examinations of many hundreds of samples of the Washington milk supply, laboratory tests to assist in the diagnosis of the disease, and examinations of specimens in a search for bacillus-carriers, etc.

Thus far our studies indicate that typhoid fever will cease to be a "problem" in any community having clean water, an uninfected milk supply, and in which cases of the disease are treated as dangerous and contagious.

Certain improvements in the situation should be noted. The shallow wells have been closed, and during the summer of 1907 filtered water of good sanitary quality was supplied the city of Washington. Bills have been introduced into Congress with the object of improving the milk supply. The sewerage system has been practically completed. Laws are being enforced for the condemnation of insanitary dwellings, and to effect other improvements in the general sanitary conditions.

The case and death rates for typhoid fever in the District of Columbia in 1907 were lower than recorded for any previous year.

The improvement in the typhoid fever situation within certain limits should go on from year to year. The price of such improvement, however, will be well directed and unceasing efforts on the part of the local authorities, with the cooperation of the medical profession. The water must remain of good quality, the market milk must be improved, and a greater regard must be had for the contagious nature of the disease.

Attention is called to certain modifications in chart No. 4 of Bulletin No. 35, rendered necessary by a reexamination of the records of the health office. Chart No. 3 in this report is based upon records furnished by the health officer and gives the corrected curve of the seasonal prevalence of typhoid fever in previous years.

We have again profited by the cooperation of Dr. W. C. Woodward, the health officer of the District of Columbia, and also the officers of the filtration plant. We are indebted to the hospitals throughout the city and to many physicians for help in the epidemiological studies.

In drawing up the conclusions and recommendations of this report we have had the benefit of consultation with the advisory board of the Hygienic Laboratory, composed of eminent scientists and sanitarians. This privilege is appreciated, and we desire to acknowledge the help we have received from the members of the board collectively and individually.

M. J. Rosenau,
Director Hygienic Laboratory, Chairman.
L. L. Lumsden,
Passed Assistant Surgeon.
Joseph H. Kastle,
Chief of Division of Chemistry, Recorder,
United States Public Health and Marine-Hospital Service.

REPORT NO. 2 ON THE ORIGIN AND PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA.

(1907.)

Our study of the prevalence of typhoid fever in the District of Columbia was resumed in May, 1907, and all cases reported to the health office from May 1 to November 1 have been investigated. Six hundred and seventy-five cases were reported in this period. In regard to five of these cases no accurate information could be obtained, so they were abandoned, leaving 670 cases for consideration in this report.

DIAGNOSIS.

It was not practicable for us to study the cases in clinical detail, but judging by the histories obtained in some instances, we are of the opinion that quite a number of the cases were incorrectly diagnosed and should not have been placed on the records as cases of typhoid fever. However, as the percentage of error in diagnosis was probably no greater this year than in previous years, and in order that our figures for this year should be comparable to those for previous years, we have included in our epidemiological studies every case reported as typhoid fever by the attending physician.

With a view to forming some idea of the amount of error in diagnosis, we examined the blood of 27 cases reported as typhoid fever between March 4 and December 2, 1907. Five cubic centimeters of blood were withdrawn from an arm vein and planted at once into 10 c. c. of sterilized ox bile. This was incubated for 24 hours, and subcultures made upon Endo and other media. This part of the work was done for us by Passed Assistant Surgeon A. M. Stimson, in the Division of Pathology and Bacteriology of the Hygienic Laboratory.

The following is a summary of the results:

Number of specimens examined	27
Specimens in which *B. typhosus* was found	8
Specimens in which *B. paratyphosus* A was found	1
Total	9 = 33.3 per cent.

Cases from which blood was taken in first week	7
Positive	2 = 28.5 per cent.
Cases from which blood was taken in second week	8
Positive	2 = 25.0 per cent.
Cases from which blood was taken in third week	4
Positive	3 = 75.0 per cent.
Cases from which blood was taken in fourth week	3
Positive	2 = 66.6 per cent,
Cases in which the time of disease was not stated	5
Positive	0 = 0.0 per cent.

Of the 27 cases examined probably a definite proportion were not typhoid at all, for many of these tests were made at the request of physicians in doubtful cases to clear up difficult diagnoses. Three of the cases, after being reported, were determined clinically not to have been typhoid fever. Further, it was not practicable to make more than one examination from each case. We now know that 5 c. c. of blood is not always sufficient to demonstrate the presence of the typhoid bacillus at a single examination. In one of our cases 10 c. c. of blood was taken and divided into two portions. One portion remained sterile, while the other gave a pure culture of the typhoid bacillus.

In each instance the identity of the bacillus was determined by complete cultural and agglutinative tests.

Excluding the three cases clinically determined not to have been typhoid, the percentage of instances in which the bacillus was recovered is brought up to 37.5 per cent. Judging by the results of other investigators, the method followed in making these blood examinations should have demonstrated the typhoid bacillus in a larger percentage of instances if all of the 27 cases had been typhoid fever.

The number of cases of typhoid fever in the District of Columbia which are unrecognized and not reported probably exceeds considerably the number reported as typhoid fever under mistaken diagnosis. Thus, error in diagnosis alone makes it impossible by epidemiological investigations to account for a considerable number of cases. All data obtained in regard to cases reported as typhoid fever but which are not are misleading and so obscure the general problem. Spread of infection directly by contact or indirectly by persons, flies, milk, etc., from cases unrecognized and unreported cannot be determined, especially in a large community.

From the standpoint of prophylaxis, the unrecognized cases are, of course, more important than the cases erroneously reported as typhoid fever. In order that the actual prevalence of typhoid fever in a community may be determined and that proper measures to prevent the spread of the disease may be carried out, the correct and early diagnosis of all cases is of fundamental importance.

TYPHOID FEVER CASE CARD.

In our study of the cases the following form was used, and all facts called for by this form were carefully investigated for each case. This form varies somewhat from that used last year.

Public Health and Marine-Hospital Service—Hygienic Laboratory.

TYPHOID FEVER CASE CARD.

Date of investigation, ———. Case No. ——.
Name, ———.
Age, ——. Color, ———. Sex, ———. Nationality, ———.
Probable date of onset, ———. Date definite symptoms, ———.
Name and address of physician:

RESIDENCE.

Resident of District of Columbia, —— years, —— months.
Residence when taken sick, ———; from ——— to ———.
Previous residences, ———; from ——— to ———.
Subsequent residence, ———; from ——— to ———.
Temporary absences from District of Columbia within 30 days prior ———.
Number of occupants, ———. Ages, ———.
Number of occupants who have had typhoid, ——. When, ———.
Newcomers in house within three months prior, ——.
Newcomers in house had typhoid? ———.
Servants, ——
  White—
    Resident, ——. Typhoid? ———.
    Nonresident, ——. Typhoid? ———.
  Colored—
    Resident, ——. Typhoid? ———.
    Nonresident, ——. Typhoid? ———.
Typhoid at homes of servants, ———. When? ———.
Disposal of sewage, ———. Water-closets in house? ——.
Water-closets in yard? ——. Privy? ——. Location, ———.
General sanitary condition of residence, ———.

OCCUPATION.

Place, ———; from ——— to ———.
Other cases:

WATER WITHIN THIRTY DAYS PRIOR.

——— solely; ——— principally.
——— occasionally.

FOOD WITHIN THIRTY DAYS.

Where taken, ———.
Milk (how used), ———; from ———.
  Boiled? ———. Pasteurized? ———.
Ice cream? ———. Where? ———.
Uncooked fruits and vegetables, ———.
Shellfish, ———.

CONTACTS.

Association 30 days prior with patients in febrile stage, ———.

Association with suspected cases, ———.

Association with persons who have had typhoid within 6 months, ———; 1 year, ———; 2 years, ———; 3 years, ———; 4 years, ———; 5 years, ———.

Association 30 days prior with persons in contact with patients in febrile stage, ———.

Treatment of stools and urine of patients, ———.

Other precautions, ———.

Remarks: ———.

Summary: ———.

——— ———.

(Signature of investigator.)

IMPORTED CASES.

In determining the number of cases in which the infection was contracted out of the District of Columbia the same method, but with a little more detail, was adopted as in our study of the cases reported from June 1 to November 1, 1906, and as published in our previous report.[a]

The cases fall under the following heads:

	Cases.
(*a*) Infection undoubtedly contracted out of the District of Columbia	117
(*b*) Infection probably contracted out of the District of Columbia	30
(*c*) Infection contracted in or out of the District of Columbia, chances about equal	40
(*d*) Infection undoubtedly contracted in the District of Columbia	363
(*e*) Infection probably contracted in the District of Columbia	120
Total	670

Considering the cases on the percentage of chances of the infection having been contracted out of the District of Columbia, they are as follows:

		Cases.
(*a*)	117 cases, chances 100 per cent	117
(*b*)	30 cases, chances 75 per cent	22
(*c*)	40 cases, chances 50 per cent	20
(*d*)	363 cases, chances 0 per cent	0
(*e*)	120 cases, chances 12.5 per cent	15
	670 cases. Total	174

On this estimate, about 26 per cent of the cases investigated contracted the infection out of the District of Columbia, as against about 15 per cent of the cases studied by us in 1906.

In estimating the chances of contracting the infection out of the District, the time element was given the greatest consideration, but the prevalence of typhoid fever in the communities visited and known exposure of the person to infection while away were also taken into account. It should be borne in mind that the number of imported cases in a city is probably wholly or partly offset by the number of cases which contract the infection in the city and develop the disease elsewhere.

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[a] Bulletin No. 35, Hygienic Laboratory, P. H. and M. H. S. Report on the Origin and Prevalence of Typhoid Fever in the District of Columbia, 1906.

No detailed epidemiological investigations were made of the cases which were considered as certainly or almost certainly having contracted the infection out of the District. Therefore, most of the data in this report refer to the 523 cases comprised under *c*, *d*, and *e* of the above classification.

SEX.

Of the 523 cases, 304 occurred in males and 219 in females. Males compose about 48 per cent and females about 52 per cent of the population of the District of Columbia: 58.12 per cent of the cases were among males, showing, as was the case in the group studied by us in 1906, a relatively slightly higher typhoid fever morbidity rate among the males.

Two conditions, perhaps, contribute to the higher rate among the males; first, due to habits of occupation, etc., men are more exposed to infection, and, secondly, during the summer months, when the disease is most prevalent in Washington, a relatively larger number of women than men leave the city.

RACE.

Three hundred and eighty-seven, or 74 per cent, of the cases were among whites, and 136, or 26 per cent, among negroes. According to the police census for the spring of 1907, the population of the District of Columbia consists of 233,403 whites and 96,188 negroes, a total of 329,591. Thus negroes compose 29.18 per cent of the population, while the cases of typhoid fever among them composed 26 per cent of the total number investigated, showing a relatively somewhat lower morbidity rate among negroes than among whites. A discussion concerning the marked decrease in the morbidity and mortality rates in the negroes during the calendar year 1907 will be found on page 22, under "Prevalence."

AGES.

The following table gives the cases classified by age and sex, along with the population of the District of Columbia in 1900 (U. S. Census Report):

Ages, years.	Number of cases.			Population, 1900.			Ratio of cases to population.
	Male.	Female.	Total.	Male.	Female.	Total.	
0-4	11	11	22	11,683	11,467	23,150	1-1,052
5-9	48	33	81	11,708	12,023	23,731	1- 293
10-14	47	50	97	10,953	11,781	22,734	1- 234
15-19	41	33	74	11,362	13,452	24,814	1- 335

Ages, years.	Number of cases.			Population, 1900.			Ratio of cases to population.
	Male.	Female.	Total.	Male.	Female.	Total.	
20-24	51	28	79	13,774	17,736	31,510	1- 398
25-29	44	27	71	13,345	16,410	29,755	1- 419
30-34	27	13	40	11,911	12,857	24,768	1- 619
35-39	17	10	27	10,409	11,472	21,881	1- 810
40-44	3	4	7	8,679	9,255	17,934	1-2,562
45-49	8	8	16	6,876	7,750	14,626	1- 914
50-54	2	0	2	6,371	6,928	13,299	1-6,649
55-59	2	1	3	5,285	5,001	10,286	1-3,428
60-64	2	0	2	4,123	4,078	8,201	1-4,100
65-69	0	0	0	2,431	2,604	5,035	-------
70-74	1	1	2	1,611	1,829	3,440	1-1,720
75-79	0	0	0	796	1,049	1,845	-------
80-84	0	0	0	351	567	918	-------
85-89	0	0	0	132	206	338	-------
90-94	0	0	0	26	73	99	-------
95-99	0	0	0	10	29	39	-------
100 and over	0	0	0	6	14	20	-------
Age unknown	0	0	0	162	133	295	-------
Total	304	219	523	132,004	146,714	278,718	-------

The youngest person affected was 2 years and the oldest 73 years of age. As the cases occurred in 1907 and the census was held in 1900, the figures are not strictly comparable, but serve as a fair basis for an estimate. It will be noted that the largest number of cases proportionate to population occurred among persons from 10 to 14 years of age, and the next largest among persons from 5 to 9. Thus, our studies for the two years show that the opinion usually held that typhoid fever is most prevalent among persons in early adult life (20 to 30 years) is not true for the District of Columbia, the disease here being most prevalent among children. As typhoid fever in children is frequently mild and irregular, many cases are not recognized. Therefore it is probable that more cases than the table indicates actually occur in persons under the age of 15 years.

By decades, 103 of the cases were under 10 years, 171 between 10 and 20, 150 between 20 and 30, 67 between 30 and 40, 23 between 40 and 50, and 9 over 50.

The rather large proportion of cases among children points especially to two factors in the transmission of the infection of typhoid fever—contact and milk. Large numbers of children from many households are in free and intimate association in playing, so that the chances of spread of infection among them by direct or indirect contact must be greater than among adults.

Furthermore, mild and irregular attacks of typhoid fever are known to occur with greater frequency in children than in adults, so that the spread of infection by contact from unrecognized cases among children must be correspondingly more frequent.

Children drink more milk than adults, so that where milk is a frequent source of infection a relatively large number of cases among children may be expected.

In considering the number of cases among children in a community where typhoid fever has prevailed extensively for a number of years, we have to take into account that a proportionately larger number of adults have been rendered partially or completely immune by previous infection.

Contact between child and child and between child and adult is of a close and intimate nature, and such as to favor the spread of the infection of typhoid fever. Considering this fact, along with the known extensive prevalence of the disease among children, we have a possible explanation of the spread of the infection to many of the cases unaccounted for by epidemiological investigations, thus robbing the general problem of some of its mystery. Koch found that 49 of the 64 unrecognized cases of typhoid fever at Trier occurred among children. (Die Bekämpfung der Typhus, Berlin, 1903.) The large number of cases among children emphasizes the importance of keeping this factor in mind in carrying out practical measures to prevent the spread of the infection.

NATIONALITY.

In the following table is given the nationality of the cases along with the number of foreign born in the District of Columbia for 1900, according to the United States Census reports. For some of the nationalities the population no doubt has changed considerably since 1900, so that the figures in the table are not strictly comparable.

Nationality.	Number of cases.	Number of foreign-born in District of Columbia, census of 1900.
American	495	
Danish	1	88
English	3	2,299
German	5	5,857
Hungarian	1	48
Irish	5	6,220
Italian	11	930
Russian	1	807
Swedish	1	234
Total	523	

LENGTH OF RESIDENCE.

The length of residence in the District of Columbia of the persons affected was as follows:

Length of residence:	Cases.
Under six months	20
6 months to 1 year	15
1 to 2 years	28
2 to 3 years	16
3 to 4 years	19
4 to 5 years	17
5 to 10 years	62
10 to 15 years	26
15 to 20 years	16
20 to 30 years	18
30 to 40 years	7
40 to 50 years	4
50 to 60 years	1
Life	239
Not determined	35
	523

The ages of the 239 life residents of the District of Columbia affected were as follows:

Ages:	Cases.
0 to 4 years	17
5 to 9 years	55
10 to 14 years	67
15 to 19 years	38
20 to 24 years	23
25 to 29 years	22
30 to 34 years	8
35 to 39 years	7
40 to 44 years	1
45 to 49 years	1
	239

Nearly 50 per cent of the cases were among persons who had resided in the District of Columbia all their lives, so the disease can not be considered to have prevailed to any very unusual extent among newcomers. However, it should be noted that the number of cases, 115, among persons who had resided in the District less than five years was nearly twice as large as the number, 62, among persons who had resided in the District for five to ten years, and over four times as large as the number, 26, among those who had resided in the District from ten to fifteen years.

In considering these figures we should take into account that the transient element of Washington's population is rather large, and that among persons who have lived in the District less than ten years there is, of course, a large proportion of children.

PREVALENCE.

The following table shows the cases according to dates of definite onset:

Day of month.	May.	June.	July.	August.	September.	October.
1	2	0	0	11	5	5
2	1	0	1	5	5	8
3	2	0	1	6	3	3
4	1	1	2	6	5	3
5	2	2	3	7	3	2
6	0	0	3	2	4	2
7	0	1	3	6	4	3
8	0	1	3	9	1	2
9	1	2	5	4	2	2
10	3	2	6	7	11	6
11	0	1	2	4	1	1
12	1	2	3	8	2	2
13	0	3	2	11	3	4
14	2	0	2	6	2	3
15	0	0	3	7	3	6
16	1	2	2	3	2	3
17	1	2	2	5	4	3
18	0	1	3	10	4	0
19	1	0	4	2	5	0
20	1	3	5	5	8	7
21	0	1	3	5	4	2
22	0	0	1	5	3	1
23	0	2	7	1	1	0
24	0	2	1	3	1	1
25	1	3	7	6	6	0
26	1	3	3	2	1	0
27	2	3	7	7	4	0
28	0	2	7	5	6	0
29	3	2	7	5	2	0
30	1	1	1	2	3	0
31	0	----------	3	1	------------	0

As in our previous report, the cases are tabulated and charted according to date of onset of definite symptoms, it having been found again that in the majority of instances accurate statements as to date of onset of the first slight symptoms could not be obtained.

The following table shows the progress of the disease as indicated by dates of onset of the cases in periods of a half month. The number of cases for corresponding periods in 1906 are placed in a parallel column:

Periods.	Cases.	
	1907.	1906.
February 1 to 15, incomplete	1	--------
April 1 to 15, incomplete	1	--------
April 16 to 30, incomplete	7	--------
May 1 to 15, complete	15	--------

Periods.	Cases.	
	1907.	1906.
May 16 to 31, complete	12	
June 1 to 15, complete	15	32
June 16 to 30, complete	27	32
July 1 to 15, complete	39	101
July 16 to 31, complete	63	131
August 1 to 15, complete	99	139
August 16 to 31, complete	67	70
September 1 to 15, complete	54	59
September 16 to 30, complete	54	41
October 1 to 15, incomplete	[a]52	98
October 16 to 31, incomplete	[a]17	22

[a] Completed figures for October, including cases reported subsequent to November 1, are: October 1 to 15, 64; October 16 to 31, 42.

The rate began to increase in the latter half of June and gradually rose through July and the early part of August, reaching its maximum about the middle of August and then rather gradually declined through the latter half of August and through September. A comparison of the rates for the summers of 1906 and 1907 shows that in 1907 the increase in the rate was somewhat later, decidedly less, and with a rise and fall more gradual than that of 1906. (See Charts Nos. 1 and 2.) The total number of cases occurring in June, July, and August, 1907, was 310, while the total for the corresponding period of 1906 was 505, a difference of 195 cases. During the winter, spring, and fall seasons the rate of prevalence for the two years was about the same. (See Table, p. 23.)

The results of our investigations suggest that the lower rate for the summer of 1907 for the most part was not due to a decrease in the operation of two known factors in the spread of typhoid fever infection, viz, milk and contact. Of the 505 cases in the summer months of 1906 there were 48, or 9.5 per cent, that occurred in the course of two pronounced milk outbreaks, and were attributed to infected milk. Of the 310 cases in the corresponding period of 1907, 28, or 9.03 per cent, occurred in the course of a pronounced milk outbreak and were attributed to milk infection.

Of the 747 cases investigated by us and considered as having contracted the infection in the District of Columbia in 1906, 7.3 per cent were fairly definitely attributable to infection by direct or indirect contact; while of the 523 cases in 1907, 19 per cent were fairly definitely attributable to infection by direct or indirect contact. Thus, so far as the results of our investigation indicate, milk as a factor in pronounced outbreaks operated relatively to about the same extent during the two summers and contact operated as a factor relatively to certainly as great an extent in the 1907 period as it did in the 1906

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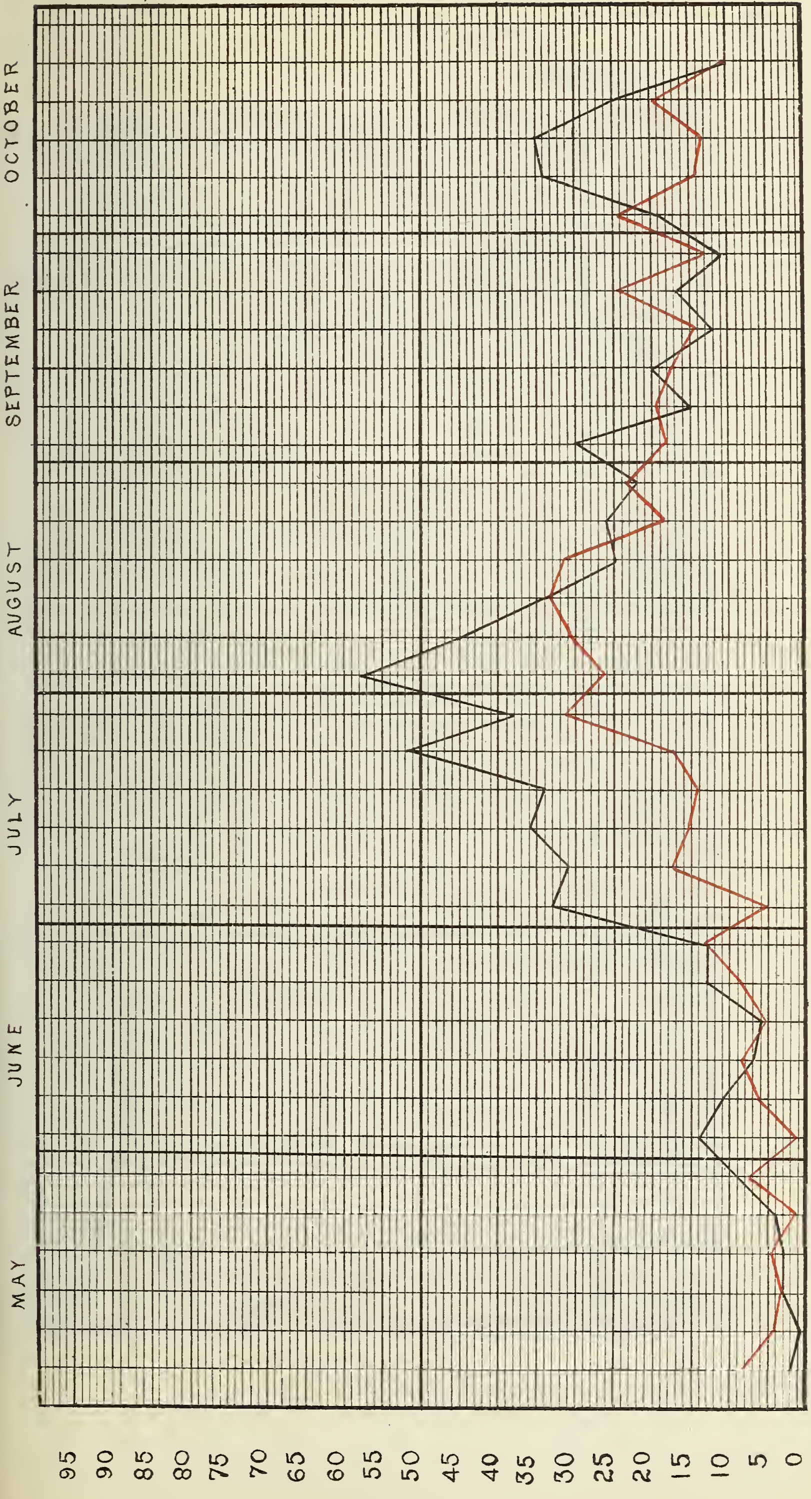
CHART No. 1

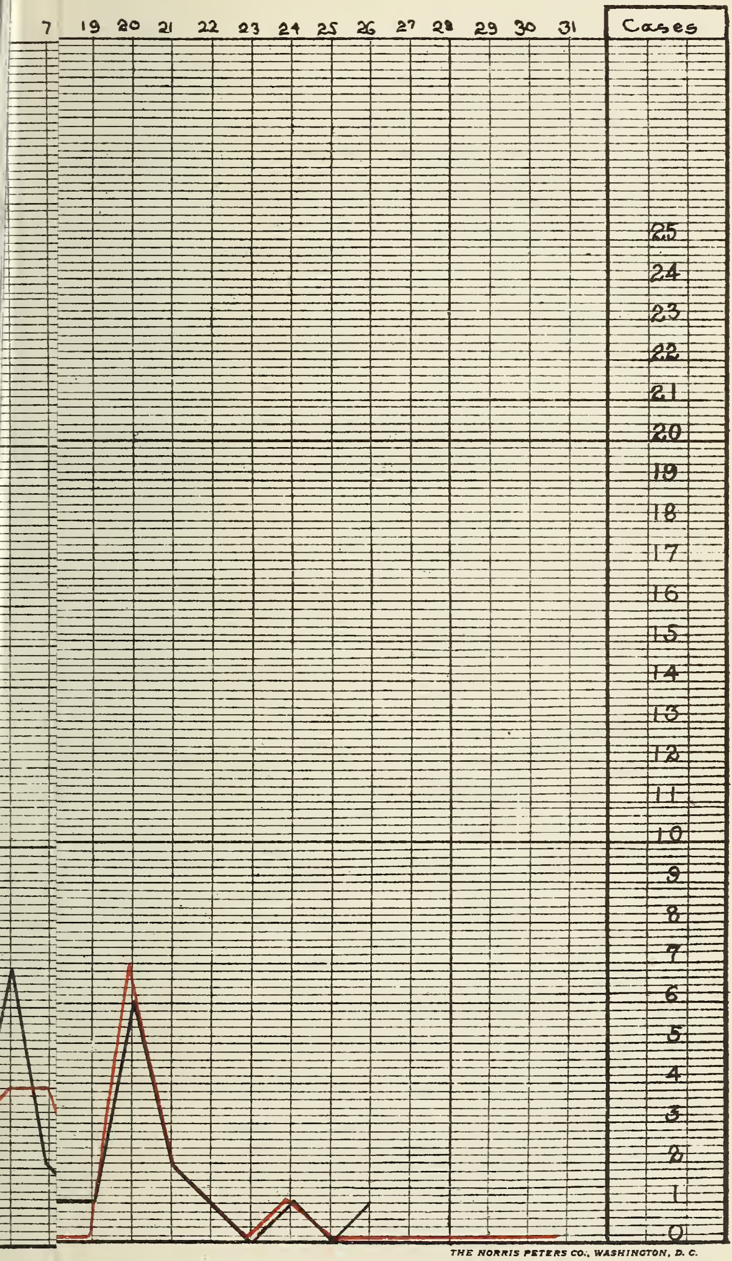


CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM JUNE 1 TO NOVEMBER 1, 1906, CHARTED ACCORDING TO THE DATE OF ONSET OF ILLNESS, IN FIVE-DAY PERIODS.

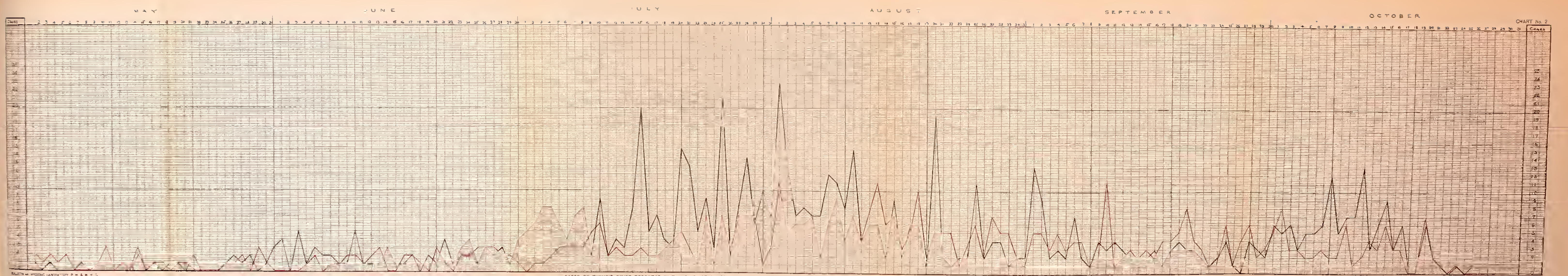
CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM MAY 1 TO NOVEMBER 1, 1907, CHARTED ACCORDING TO THE DATE OF ONSET OF ILLNESS, IN FIVE-DAY PERIODS.

THE NORRIS PETERS CO., WASHINGTON, D. C.

CHART No. 2



THE NORRIS PETERS CO., WASHINGTON, D. C.

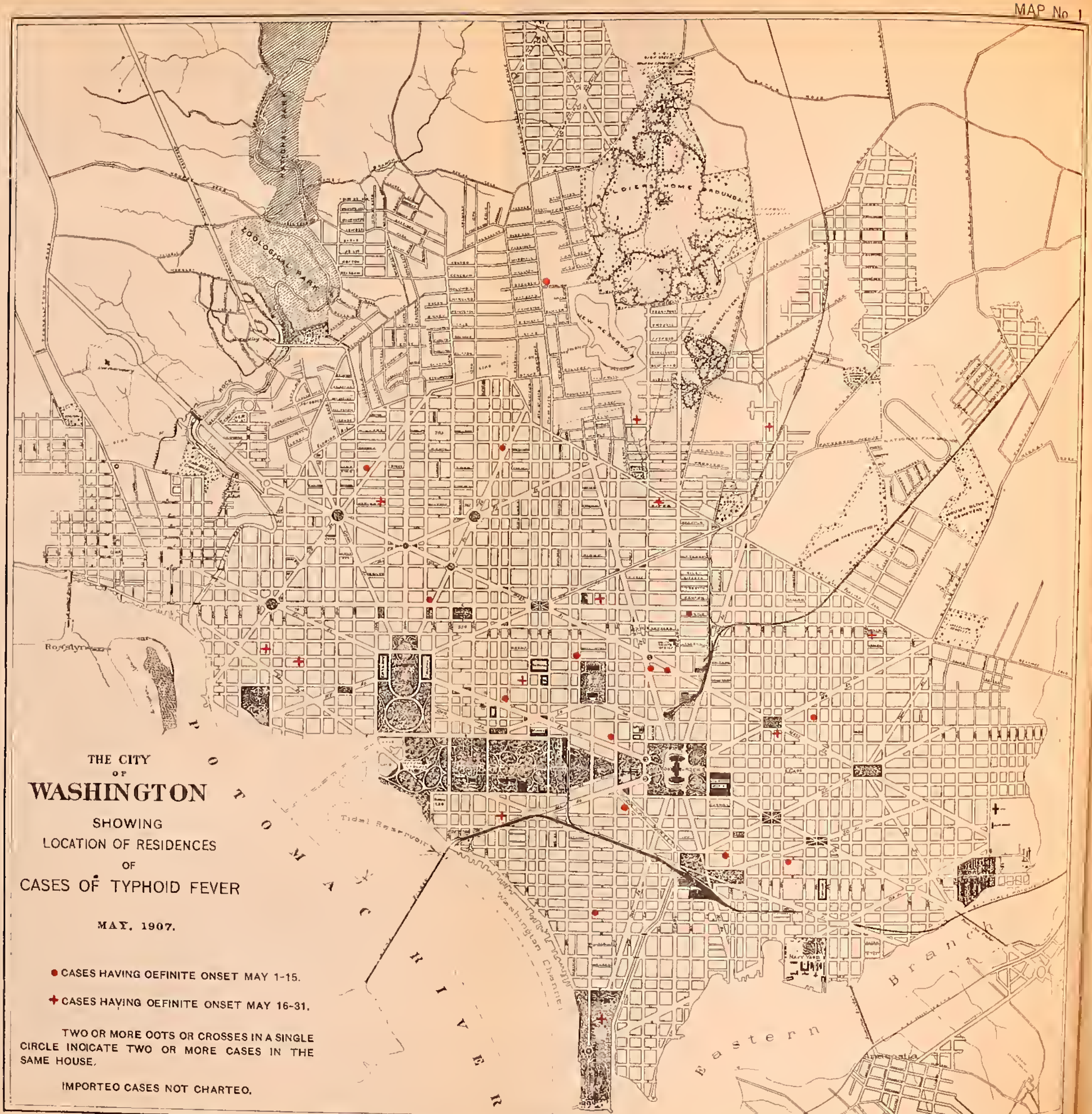


BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S.

——— CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM JUNE 1 TO NOVEMBER 1, 1906, CHARTED ACCORDING TO DATE OF ONSET OF DISEASE.

——— CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM MAY 1 TO NOVEMBER 1, 1907, CHARTED ACCORDING TO DATE OF ONSET OF DISEASE.

1
D. C.



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

MAY, 1907.

● CASES HAVING DEFINITE ONSET MAY 1-15.

✚ CASES HAVING DEFINITE ONSET MAY 16-31.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

THE NORRIS PETERS CO. WASHINGTON, D.C.

No. 2

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No. 2

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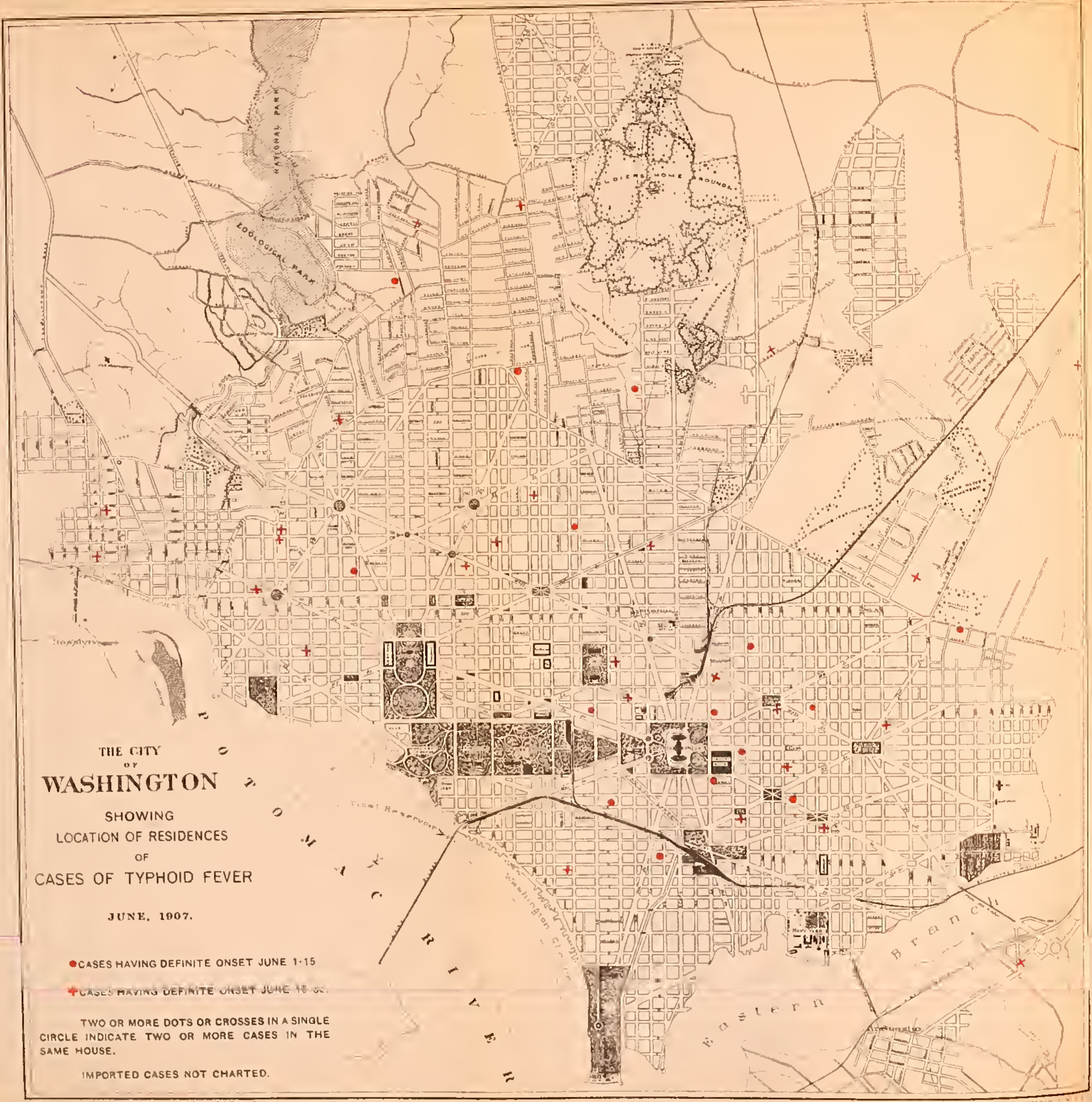
MAP No. 2



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

JUNE, 1907.

● CASES HAVING DEFINITE ONSET JUNE 1-15

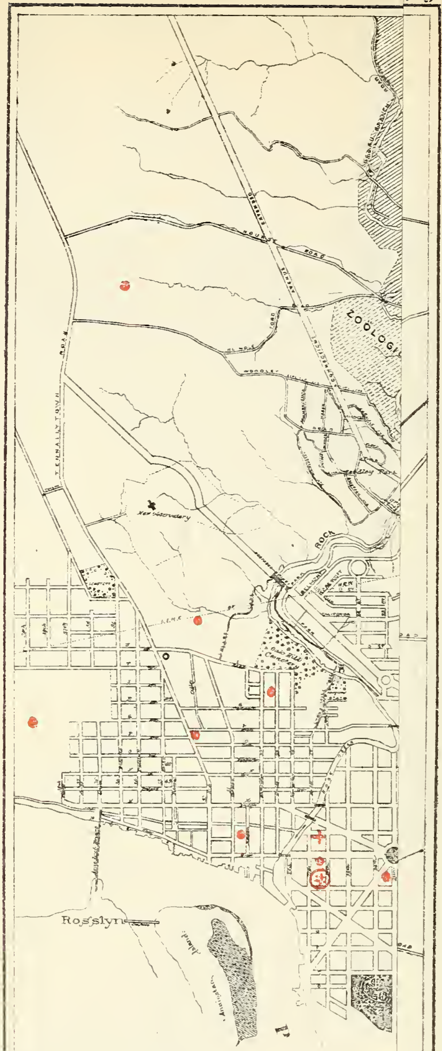
✚ CASES HAVING DEFINITE ONSET JUNE 16-30.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S.

THE NORRIS PETERS CO., WASHINGTON, D. C.



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

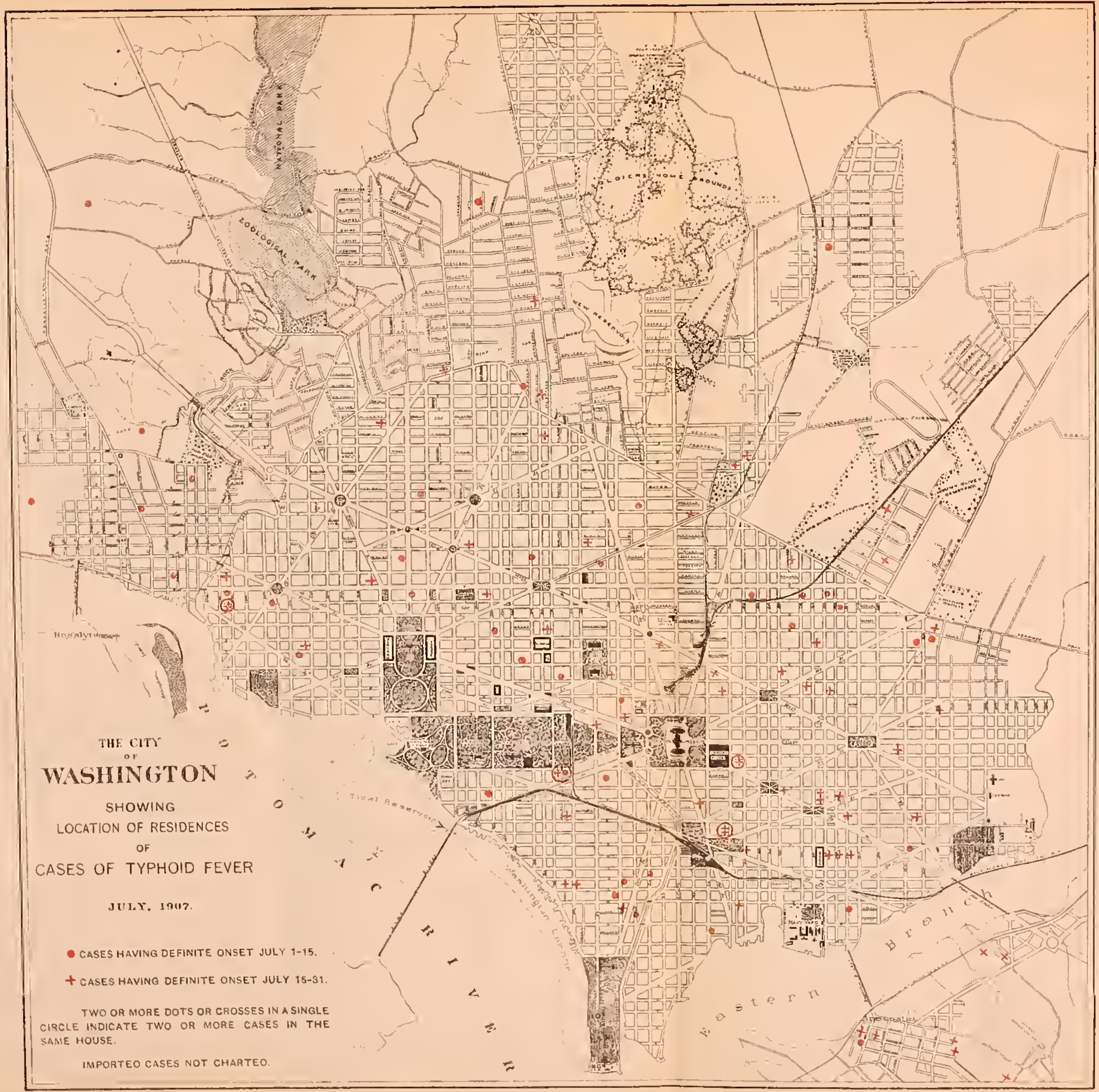
JULY, 1907.

- ● CASES HAVING DEFINITE ONSET JULY
- + CASES HAVING DEFINITE ONSET JUL

TWO OR MORE DOTS OR CROSSES IN CIRCLE INDICATE TWO OR MORE CASES SAME HOUSE.

IMPORTED CASES NOT CHARTED.

MAP No. 3



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

JULY, 1907.

● CASES HAVING DEFINITE ONSET JULY 1-15.

+ CASES HAVING DEFINITE ONSET JULY 16-31.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

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THE NORRIS PETERS CO., WASHINGTON, D. C.

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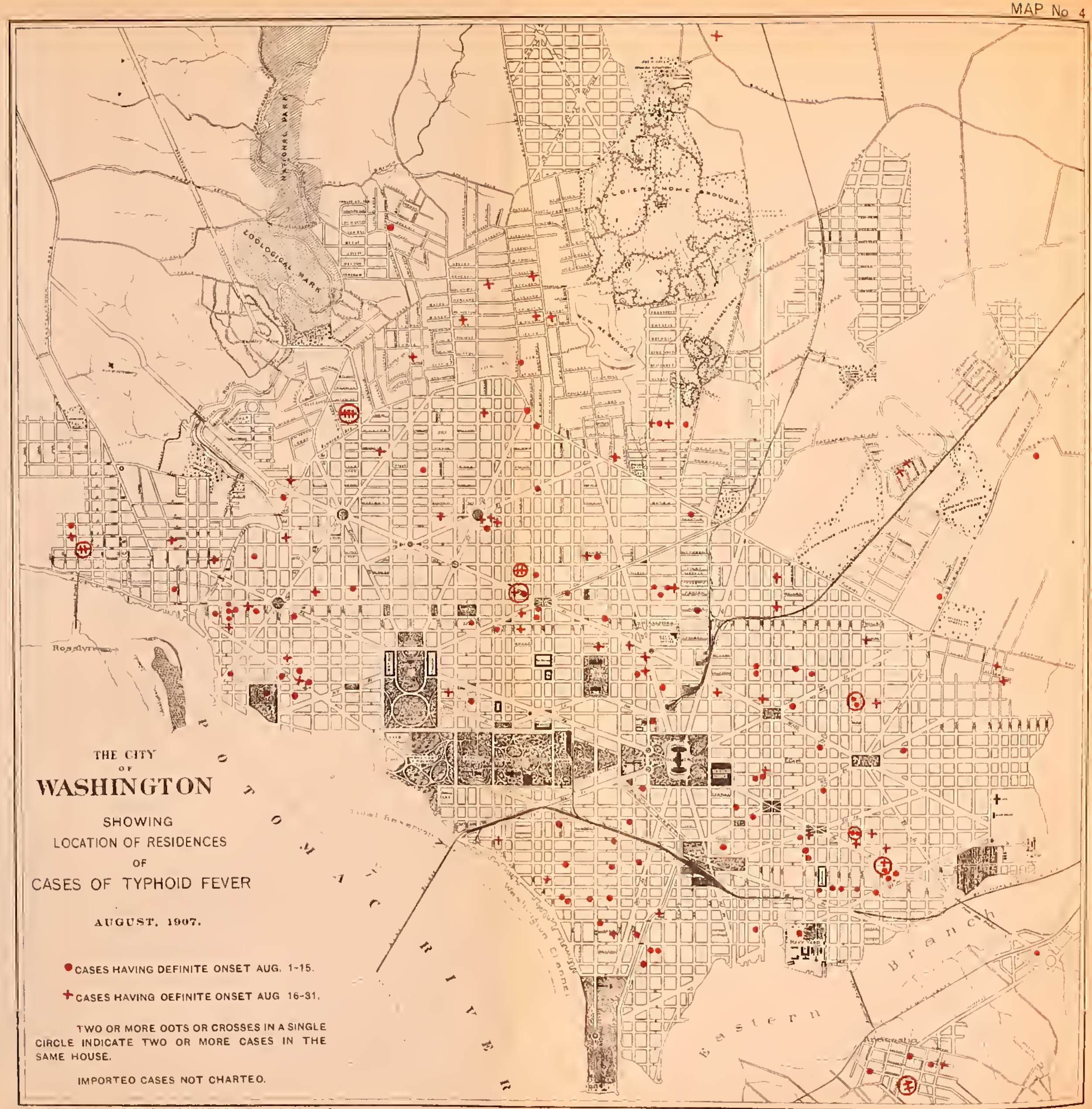
MAP No. 4



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

AUGUST, 1907.

● CASES HAVING DEFINITE ONSET AUG. 1-15.

✚ CASES HAVING DEFINITE ONSET AUG 16-31.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

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THE NORRIS PETERS CO., WASHINGTON, D. C.

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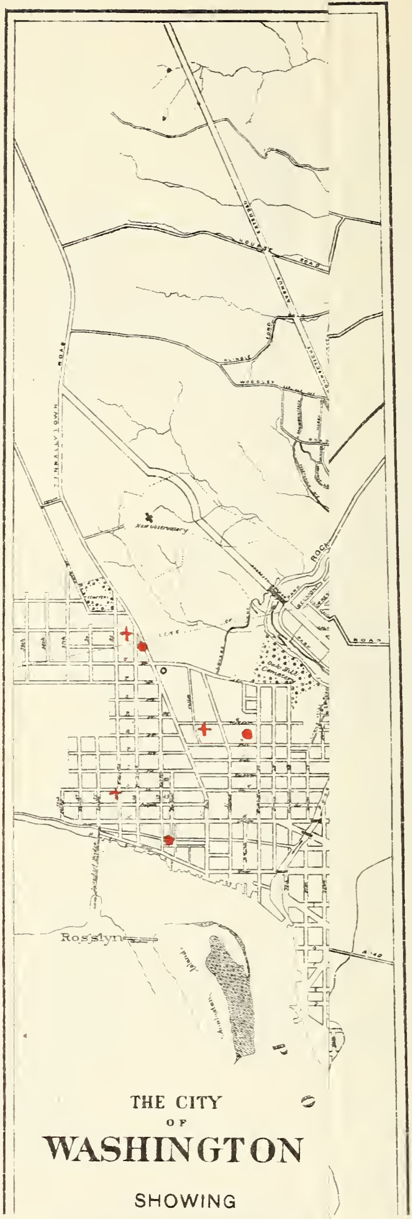
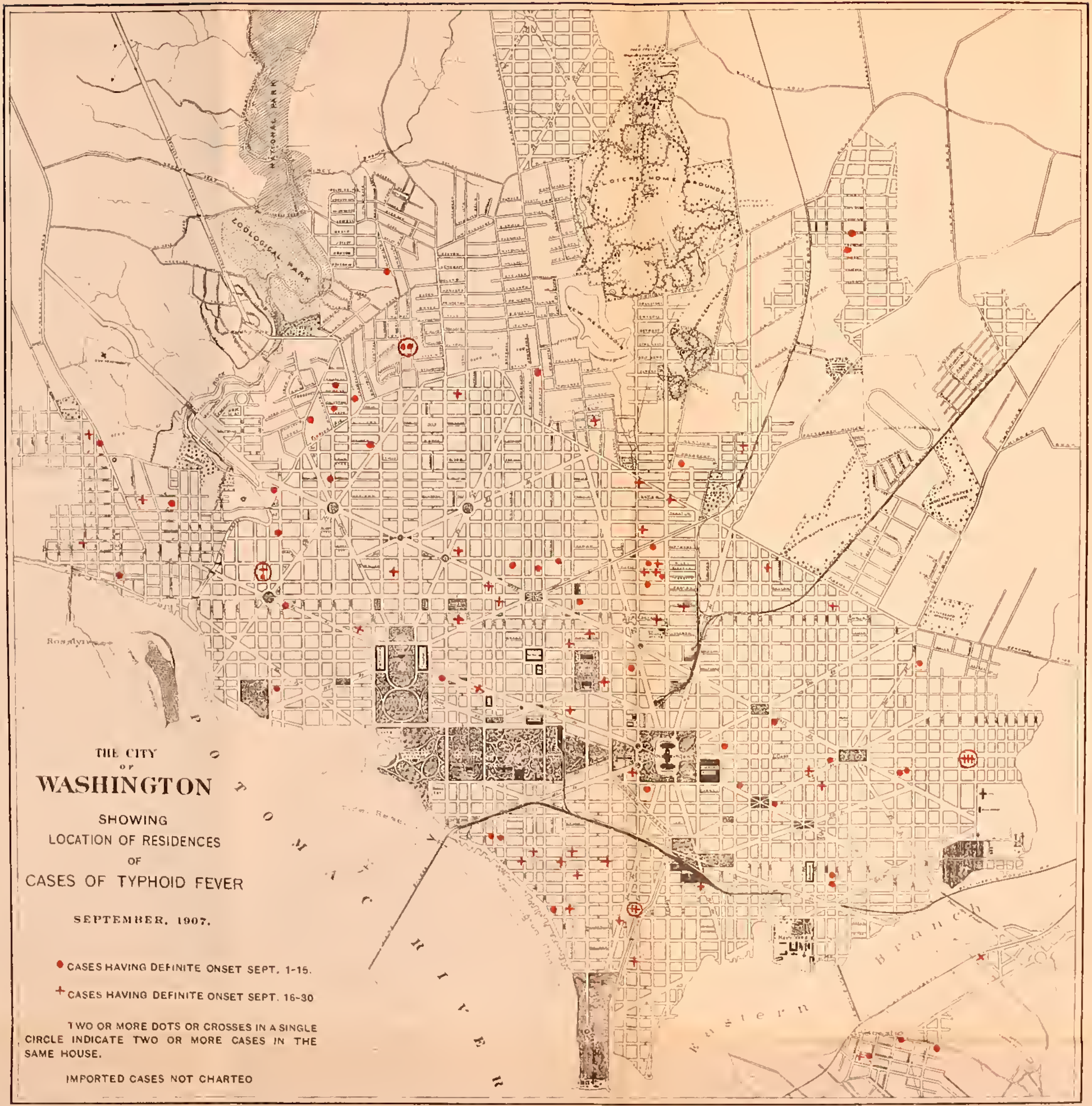


THE CITY
OF
WASHINGTON

SHOWING

MAP No. 5



THE CITY
OF
WASHINGTON

SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

SEPTEMBER, 1907.

● CASES HAVING DEFINITE ONSET SEPT. 1-15.

+ CASES HAVING DEFINITE ONSET SEPT. 16-30

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S.

period. Therefore, it appears that the difference in rate for the two summers may have been due to a difference in extent of operation of some factor or factors other than milk and contact.

That the difference in prevalence of typhoid fever in Washington for the two summers was probably not due to a difference in temperature or humidity is indicated by reports from Baltimore that typhoid fever was fully as prevalent in that city in the summer of 1907 as in that of 1906. The rise in both cities was belated in the summer of 1907.

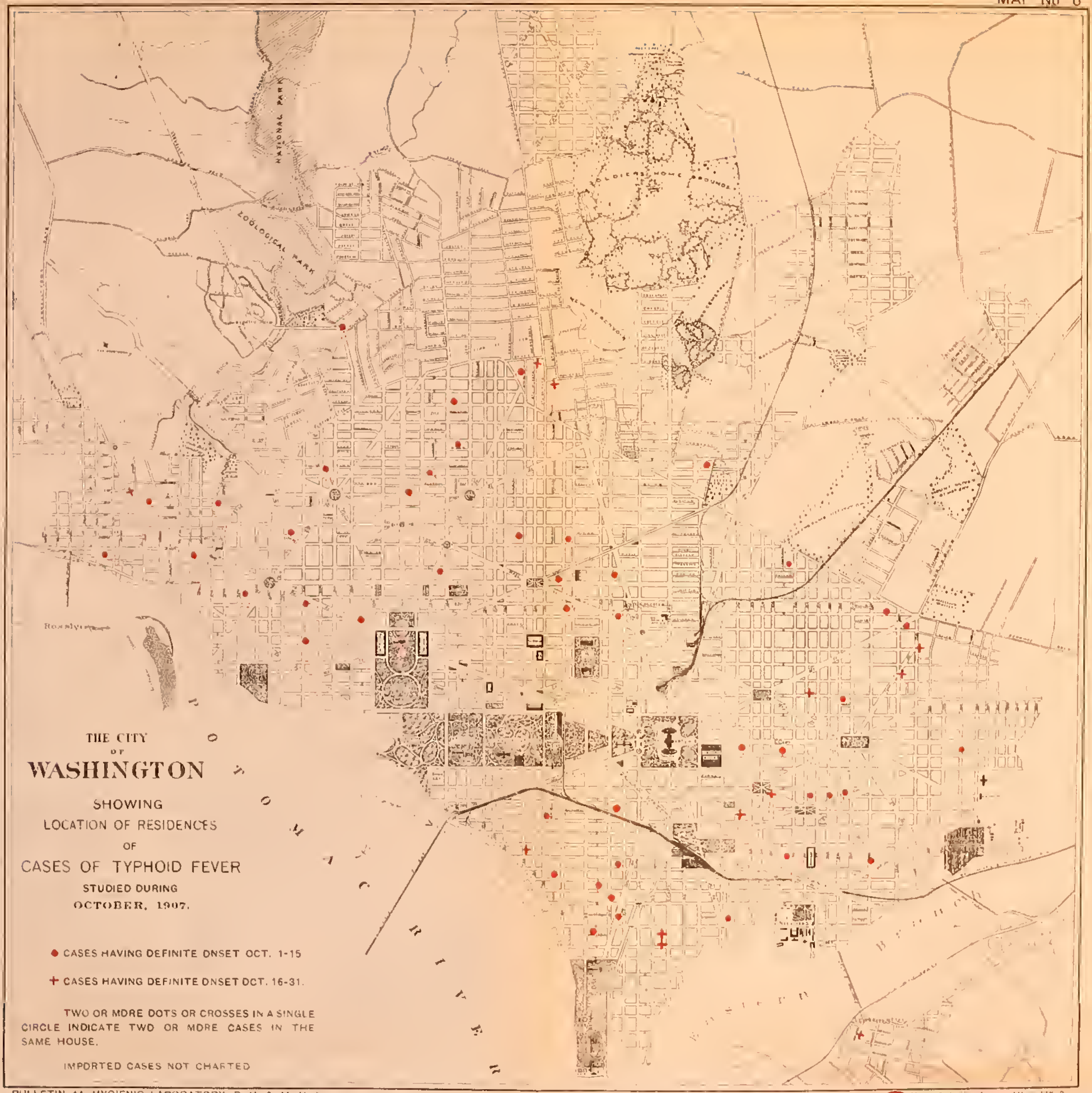
So far as can be judged by reports received from various cities there has been no material difference in the prevalence of typhoid fever throughout the country during the two years. In some places there has been more, in others less, and in some about the same during the two years.

In the 1907 period there was relatively a considerably smaller number of cases among the wealthy class of people than in the 1906 period. Maps 1, 2, 3, 4, 5, 6, and 7 show the distribution of cases by place of residence when the disease was contracted. A comparison of these maps with those showing the distribution of the cases studied in 1906 (see Bulletin No. 35) shows a somewhat less uniform distribution of cases during the half monthly periods of 1907. In 1907 there was a quite general distribution of cases throughout the city, but in proportion to population there was relatively a larger number of cases in the more unhygienic sections of the city.

Therefore, it appears from our observations this year that typhoid fever may be quite uniformly and generally scattered throughout a large city having water of good sanitary quality, so far as can be judged by our present bacteriological standards. It has been assumed that a more or less widespread and uniform distribution of an infectious disease like typhoid fever points to a common factor, such as infected water. Whether this general distribution was due primarily to contaminated water in previous months or years, or to other causes, can only be determined by future studies.

The Government Hospital for the Insane, with a population of about 3,000 but with artesian well water, pasteurized milk and generally good sanitary conditions, again this season remained free of infection. One case occurred among the employees of the institution, but this case was a man who made frequent visits to, and took meals at his home in, Washington.

The total number of cases of typhoid fever in the District of Columbia reported to the health office during the calendar year 1907 was 945 as against 1,126 for the calendar year 1906. Thus, the typhoid fever morbidity rate in the District of Columbia for the calendar year 1907 was 2.87 per 1,000 of population, or one person in



THE CITY OF WASHINGTON

SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER
STUDIED DURING
OCTOBER, 1907.

● CASES HAVING DEFINITE ONSET OCT. 1-15

+ CASES HAVING DEFINITE ONSET OCT. 16-31.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED

every 348; while in 1906 the morbidity rate was 3.45 per 1,000, or one in every 289.

The number of deaths from typhoid fever in the District of Columbia during 1907 was 114, being a death rate of 34.5 per 100,000 of population, as against 49.3 for 1906. The percentage of deaths to cases (mortality rate) for the two years was as follows:

Year.	White.	Colored.	Total.
1907	10.4	17.6	12.0
1906	10.3	24.2	14.4

Thus the mortality rate was higher in 1906 than in 1907, and as the rate for the whites was slightly lower in 1906 than in 1907, the higher rate in 1906 was due to the higher rate among the colored. As the mortality rate for the whites was about the same for the two years, it does not seem probable that the lower rate for the colored in 1907 was due to the occurrence of a milder type of infection in Washington during that year. From what we know of the facts the difference was not due to improvement in the treatment of cases in 1907. Therefore it seems that the different rates among the colored for the two years may have been due to difference in percentage of error in diagnosis and report of cases.

The mortality rates for the other three years of which there is record were as follows:

Year.	White.	Colored.	Total.
1903	10.69	19.35	12.98
1904	10.89	22.42	14.01
1905	11.66	16.33	12.94

The rate among the whites during the five years is seen to be fairly constant, while among the colored it varies considerably from year to year. Probably the lower figures more nearly approach the actual mortality rate among the negroes, and in those years in which the figures are high it is likely that the proportion of cases reported to the number which actually occurred was relatively low. This is based on the assumption that typhoid fever may not show marked variations in virulence from year to year among the colored people in an endemic area.

In the following table is given the number of cases and deaths from typhoid fever in the District of Columbia reported by months to the health office during the calendar years 1906 and 1907:

Month.	Cases.						Deaths.					
	White.		Colored.		Total.		White.		Colored.		Total.	
	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.
January	24	46	3	7	27	53	5	6	1	1	6	7
February	15	28	6	4	21	32	2	3	2	3	4	6
March	13	23	5	2	18	25	2	2	3	2	5	4
April	29	20	6	8	35	28	2	3	2	3	4	6
May	31	31	13	6	44	37	6	5	4	2	10	7
June	32	30	26	4	58	34	4	1	5	1	9	2
July	123	61	58	21	181	82	7	6	14	4	21	10
August	200	145	93	46	293	191	15	13	17	5	32	18
September	105	146	44	41	150	187	10	13	10	4	20	17
October	129	102	43	47	171	149	16	12	12	7	28	19
November	57	68	25	19	82	87	11	8	8	3	19	11
December	38	35	8	5	46	40	2	5	2	2	4	7
Total	796	735	330	210	1,126	945	82	77	80	37	162	114

As this table gives the cases by date of report of cases to the health office, which date is usually two or three weeks (and often more) subsequent to the onset of illness, and as it includes imported cases, it shows but roughly the actual progress of the disease in the District of Columbia. Table on page 19 is much more accurate in this respect. The above table shows that the number of cases and deaths from typhoid fever reported among the negroes in 1907 was much smaller than in 1906, a large part of the difference in total cases and deaths for the two years being due to the lower rate for the negroes in 1907. It is interesting to note that the disproportionately small number of colored to white cases in 1907 was among cases reported in the winter, spring, early summer, and late fall months—that is, out of the season in which typhoid fever has in the District of Columbia its greatest prevalence (by report of cases), namely, in July, August, September, and October. Thus, in 1907 there were reported in the eight months of January, February, March, April, May, June, November, and December 281 white cases and 58 colored cases, or a ratio of 1 colored to 4.8 white cases, while in the four months of July, August, September, and October there were reported 454 white and 155 colored cases, a ratio of 1 colored to 2.9 white cases.

In 1906 there was no such striking difference in the proportion of colored to white cases for the two corresponding portions of the year. Thus, in the eight months of January, February, March, April, May, June, November, and December, 1906, there were reported 239 white cases and 92 colored cases, a ratio of 1 colored to 2.4 white cases, while in the four months of July, August, September, and October, 1906, there were reported 537 white and 238 colored cases, a ratio of 1 colored to 2.2 white cases. In 1905 the ratio of colored to white cases for the first of the above periods was 1 to 2.9, and for the second period (July, August, September, and October), 1 to 2.5.

Considering these facts, it is evident that during the cool weather, or what may be called the *off season* for typhoid fever in Washington—that is, in January, February, March, April, May, October, November, and December, of 1907, the factors which operate to cause typhoid fever in the District of Columbia fell either disproportionately heavily upon the whites or disproportionately lightly upon the colored. In 1907 we investigated only the cases reported from May 1 to November 1, and as the marked disproportion in the cases reported among whites and colored was principally in the months not covered by our studies, we have no data of our own on which to base an explanation of this most remarkable occurrence.

For a discussion of seasonal prevalence in relation to water borne typhoid see page 45.

SANITARY CONDITION OF RESIDENCES.

In the following table is given the general sanitary condition of the residences at which the patients had lived when the infection probably was contracted, the condition of residences of cases studied in 1906 being placed in the parallel column:

Condition of residence.	Number of cases.	
	1907.	1906.
Good [a]	98	253
Fairly good [a]	228	243
Rather bad [a]	140	158
Bad [a]	57	92
Not determined [a]	0	1
	523	747

[a] The definitions of these terms correspond to those used in our previous report (Bulletin 35).

The percentage of cases among persons at residences of these four classes for the two periods was as follows:

Condition.	Percentage of cases.	
	1907.	1906.
Good	18.7	33.9
Fairly good	43.5	32.5
Rather bad	26.7	21.2
Bad	10.9	12.3

The proportion of cases among persons living at residences of which all the sanitary conditions were good was considerably smaller during the 1907 period than during the 1906 period; but again this year a majority (about 62 per cent) of the cases occurred among persons living at residences of good or fairly good sanitary condition.

DISPOSAL OF SEWAGE.

Of the 523 cases, 462 were among persons living at residences connected with the city sewerage system and at which there were water-closets, 56 at residences not connected with the city sewerage system and at which there were box privies, 1 at a residence from which the water-closet discharged into a subsoil cesspool, 1 at a residence having neither water-closet nor privy, and 3 at institutions not connected with the city sewerage system but having water-closets which discharged into local sewerage systems.

Of the 462 cases occurring at residences connected with the city sewerage system, 212 were at residences having water-closets in the house only, 163 at residences having water-closets in the yard only, and 87 at residences having water-closets in both house and yard. As in the 1906 period, there was no unusual prevalence of the disease among persons living at residences not connected with the city sewerage system and at which privies or other local means of disposing of sewage were in use. Thus, in the 1907 period 88.3 per cent and in the 1906 period 90 per cent of the cases were at residences connected with the city sewerage system and supplied with water-closets.

Of the 56 cases occurring at houses at which privies were in use, 15 were secondary cases and attributed to personal infection from cases in the same house or in a house nearby.

The danger of the spread of the infection by flies, etc., necessarily is greater where the excreta of the typhoid fever patients are deposited in privies, and it is therefore important to abolish privies as speedily as the extension of the city's sewerage system makes possible.

WATER.

Of the 523 cases, 492, or about 94 per cent, gave a history of having used unboiled Potomac water supplied through the regular city system as the sole, principal, or occasional source of water for drinking during the thirty days prior to the onset of illness. Of the cases investigated during the 1906 period, 96.54 per cent gave a history of having used the unboiled Potomac water.

The following table gives the source of water used for drinking during the thirty days prior to onset of illness by the 523 cases investigated:

Raw tap:	
Solely	253
Principally	187
Occasionally	52
Occasionally (?)	4
Boiled tap:	
Solely	3
Principally	13
Occasionally	2
Filtered tap:	
Solely	1
Principally	2
Occasionally	0
Public wells or springs:	
Solely	7
Principally	5
Occasionally	36
Bottled:	
Solely	0
Principally	1
Occasionally	8
Private wells or springs in the District of Columbia:	
Solely	12
Principally	29
Occasionally	15
Various sources out of District of Columbia:	
Principally	8
Occasionally	157

It is interesting to compare some of the figures in this table with those in a similar table for the cases studied by us in 1906. Thus, there is little difference in the proportion of cases among persons who used the raw tap water. In the 1907 period 4.3 per cent and in the 1906 period 1.3 per cent of the cases were among persons who had used water other than the raw tap as the sole source for drinking.

In the 1907 period, 165, or 31.5 per cent, of the 523 cases investigated were in persons who, during the thirty days prior to the onset of the disease, had used water principally or occasionally for drinking from some source outside of the District of Columbia as against 69, or only 9.2 per cent, of the cases for the 1906 period.

SHALLOW WELL WATERS.

The following list of public shallow wells closed during 1907 is given as furnished us by the health officer:

NORTHWEST SECTION.

Location.	Date closed.
Brightwood avenue, near District line	July 20, 1907
Eighth street and Barry place	July 17, 1907
Clark street and Elliott place	July 22, 1907
Massachusetts avenue, between Sixth and Seventh streets	July 17, 1907
New York avenue, between Fourth and Fifth streets	July 15, 1907
New Jersey avenue, between M and N streets	Do.
North Capitol street and Randolph place	Nov. 11, 1907
Ninth and H streets	July 15, 1907
Newton street, east of Brightwood avenue	July 20, 1907
Sixth, between F and G streets	July 15, 1907
Sixth, between Fairmont and Girard streets	July 17, 1907
Sixteenth and Corcoran streets	July 16, 1907
Seventeenth and K streets	Do.
T, between Seventeenth and Eighteenth streets	Do.
Third street and Indiana avenue	July 15, 1907
Third and L streets	Do.
Tenth and K streets	Do.
Tenth and N streets	July 17, 1907
Twelfth street and Florida avenue	Nov. 11, 1907
Twenty-eighth and O streets	July 18, 1907
Wisconsin avenue and P street	Do.
Wisconsin avenue and Q street	Do.
Wisconsin avenue and Thirty-third street	Do.
Thirty-fourth and Volta streets	Do.
Thirty-fourth street and Wisconsin avenue	July 19, 1907
Thirty-fifth and Reservoir streets	Do.
Thirty-second, north of Q street	July 18, 1907

NORTHEAST SECTION.

E, between Eighth and Ninth streets	July 20, 1907
Eighth and A streets	July 19, 1907
Eleventh and F streets	Do.
Fourth and E streets	Do.
Keating, between Lincoln road and First street	July 18, 1907
North Capitol, between B and C streets	July 17, 1907
South Dakota avenue and Vista street	July 24, 1907
Second and G streets	July 18, 1907
Sixth and C streets	Do.
Third and D streets	July 17, 1907

SOUTHEAST SECTION.

Eighth and D streets	July 20, 1907
Eleventh street and South Carolina avenue	July 18, 1907
Fillmore and Jackson streets	July 19, 1907
Fourth and C streets	July 16, 1907
Fourth street and South Carolina avenue	July 18, 1907
Fifth and G streets	Do.

SOUTHEAST SECTION—Continued.

Location.	Date closed.
I, between Eleventh and Twelfth streets	July 23, 1907
Jefferson, between Monroe and Fillmore streets	July 19, 1907
L, between Thirteenth and Fourteenth streets	July 22, 1907
Ninth and E streets	Do.
O, between Half and First streets	July 24, 1907
Seventh and B streets	July 23, 1907
Seventh street, between North Carolina avenue and C street	July 16, 1907
Seventeenth and Harrison streets	July 19, 1907
Third and C streets	July 16, 1907
Third street and Pennsylvania avenue	July 17, 1907
Thirteenth, between D and E streets	July 23, 1907

It will be noticed that the closing of these wells occurred too late to account for the improvement in the situation this season.

The following is a list of shallow wells in the District of Columbia still in operation, and, excepting the one on Quincy street, between Twelfth and Thirteenth streets NE., all are out of immediate touch with the public water-main system. There are various shallow wells at public schools of which there is no record at the health office, and they are not included in this list:

Northwest.—Massachusetts avenue, extended, near Wisconsin avenue; Tenleytown road, Tenleytown.

Northeast.—Benning cross roads; Quincy street between Twelfth and Thirteenth streets.

Southeast.—Elvan avenue, west of Stanton avenue; Hamilton road, near Jewish cemetery; Stanton avenue and Pomeroy avenue; Twentieth and Joliet streets, Garfield; T street hill, Hillsdale.

BACTERIOLOGIC EXAMINATION OF THE POTOMAC WATER SUPPLY.

This season the bacteriologic examination of water was confined to that supplied from the Potomac River, this now being almost the sole supply of water for the city of Washington.

The examinations were made in the Division of Pathology and Bacteriology of the Hygienic Laboratory by Passed Assistant Surgeons George W. McCoy and A. M. Stimson and Assistant Surgeon W. W. Miller. As in the previous year, the technique recommended by the committee on standard methods of water analysis of the American Public Health Association on January 9, 1905, was followed. From May 15 to November 30 two samples of water were obtained and examined daily, except Sundays. One sample was taken from the inlet at Dalecarlia Reservoir, this representing the Potomac River water as it is introduced into the first storage reservoir, and one sample from the outlet of the filtered-water reservoir, this representing the mixed effluents from all the filter beds and being the water just before it passes from the reservoir to the conduits for distribution to the city.

In the following tables are given the results of the bacteriologic examinations of samples of the raw river water and of the filtered water:

THE RAW RIVER WATER.

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir.

[Daily examinations made by the (*a*) Laboratory of the Filtration Plant; (*b*) Bureau of Plant Industry of Agricultural Department; (*c*) Hygienic Laboratory, Public Health and Marine-Hospital Service.]

MAY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
May 15	900	410	1,700	....	+(*c*) (*a*)	+(*a*) (*b*) (*c*)	−	+	+
16	800	240	1,800	....	+(*c*)	+(*a*) (*b*) (*c*)	−	+	+
17	460	515	1,080	....	+(*c*)	+(*a*) (*b*) (*c*)	−	+	+
18	210	110	520	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	−	+	+
19	....	....	....	....	....	....	....	....	....
20	850	122	1,000	....	+(*a*) (*c*)	+ (*a*) (*c*)	−	+	+
21	1,300	372	1,720	....	+(*c*)	+(*a*) (*b*) (*c*)	−	+	+
22	850	188	1,850	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
23	300	240	460	....	+(*a*)	+(*a*) (*b*) (*c*)	−	−	+
24	280	340	580	....	+(*a*) (*b*)	+(*a*) (*b*) (*c*)	−	−	+
25	550	175	280	....	....	−(*a*) (*b*) (*c*)	−	−	+
26	....	....	....	....	....	....	....	....	....
27	550	268	290	....	+(*c*)	+ (*b*) (*c*)	−	+	+
28	700	184	470	....	+(*a*) (*c*)	+ (*a*) (*b*)	−	+	−
29	750	84	580	....	+(*a*) (*c*)	+ (*a*) (*c*)	−	+	+
30	....	....	....	....	....	....	....	....	....
31	650	195	460	....	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	−	+	+
Average	653+	245+	913+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

JUNE.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
June 1	420	61			+(*a*) (*b*)	+(*a*) (*b*)	+	+	+
2									
3	9,000	327	12.600	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
4	12,000	317	14.300	+(*c*)	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+	+	+
5	8,000	1.145	11.200	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
6	3,100	966	7.350	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
7	3,600	666	5,800	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
8	1,700	535	300		+(*b*) (*c*)	+(*a*) (*b*) (*c*)	−	+	+
9									
10	550	223	3,500		+(*a*) (*b*)	+(*a*) (*b*) (*c*)	−	−	*x*
11	550	152	2,200	+(*c*)	+(*b*) (*c*)	+(*b*) (*c*)	+	+	+
12	1,200		6,650	+(*c*)	+(*c*)	+(*c*)	+	+	+
13	1,000	1,830	800	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
14	600	648	4.700	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
15	490	850	3.550	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
16									
17	700	281	7.000	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
18	3.000	630	3,900	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
19	1.100	270	1.750	+(*a*) (*b*)	+(*c*) (*a*) (*b*)	+(*c*)	−	+	+
20	600	196	830		+(*c*)	+(*b*) (*c*)	−	+	+
21	450	120	500		+(*c*)	+(*a*) (*b*) (*c*)	−	+	+
22	150	108	470		+(*a*) (*b*)	+(*a*) (*b*) (*c*)	+	+	+
23									
24	4.200	160	10.700	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
25	1.800	500	3.200	+(*a*) (*c*)	+(*b*) (*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
26	2,900	340	3.400	+(*c*)	+(*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
27	440	678	1.550		+(*a*)	+(*b*) (*c*) (*a*)	−	−	+
28	300	500	1.900				−	+	+
29	270	100	2.100		+(*b*) (*c*)	+(*b*) (*c*)	−	+	+
30									
Average	2.324+	483+	4,593+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

JULY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
July 1	1,600	131	5,600	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
2	1,900	84	2,950	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
3	1,200	202	150		+(*c*)	+(*b*)(*c*)	+	+	+
4									
5	920	162	350		+(*c*)	+(*c*)	—	+	+
6	370	97	350		+(*c*)	+(*b*)(*c*)	—	+	+
7									
8		132	960		+(*c*)	+(*c*)	—	+	+
9	130	748	2.050	+(*c*)	+(*c*)	+(*b*) (*c*)	+	+	+
10		678	2.100	+(*a*) (*c*)	+(*c*) (*a*)	+(*b*)(*c*)(*a*)	+	+	+
11	470	1,015	1,305		+(*c*)	+(*c*)	—	+	+
12	600	444	1.150	+(*c*)	+(*c*)	+(*b*)(*c*)	+	+	+
13	2,800	433	4,700	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
14									
15	2,800	189	2,800	+(*a*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	—	+	+
16		1,100	1,750	+(*b*)	+(*b*)(*c*)	+(*b*)(*c*)	—	+	+
17	500	460	2,150	+(*b*)(*c*)	+(*c*)	+(*b*)(*c*)	+	+	+
18	1.050	253	400	+(*c*)	+(*b*)	+(*b*)(*c*)	+	—	+
19	3,400	401	2,500	+(*c*)	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
20	550	125	850	+(*a*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
21									
22	7,400	137	2,400	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*c*)	+	+	+
23	15,400	1,400	1,200	+(*c*)	+(*b*)(*c*)	+(*b*)(*c*)	+	+	+
24		134	950	+(*c*)	+(*b*)	+(*a*)(*b*)(*c*)	+	—	+
25	1.800	148	500	+(*c*)	+(*c*)	+(*c*)	+	+	+
26	410	160	1,050			+(*b*)(*c*)	—	—	+
27	1.040	32	4,400		+(*b*)(*c*)	+(*c*)	—	+	+
28									
29	9,300	22	750	+(*b*)		+(*a*)(*c*)	—	—	+
30		45	550		+(*c*)	+(*a*)(*c*)	—	+	+
31		426	700		+(*c*)	+(*b*)(*c*)	—	+	+
Average	2,682	352+	1,715+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

AUGUST.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Aug. 1		35	3,000			+(*a*) (*b*)	−	−	−
2	12,200	92	180			+(*a*)	−	−	−
3	220	46							
4									
5	720	26	400		+(*c*)	+(*c*)	−	+	+
6	2,500	21	200			+(*a*) (*b*) (*c*)	−	−	+
7	1,300	112	220		+(*a*)	+(*a*) (*b*)	−	+	+
8	2,300	49	200				−	−	+
9	2,200	65	260			+(*b*)	−	−	−
10	31,400	63	50,000	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
11									
12	2,800	48	25,000		+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
13	760	45	2,450		+(*b*) (*c*)	+(*b*) (*c*)	+	+	+
14	4,500	133	16,900		+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
15	1,200	200	700			+(*a*)	−	+	+
16	550	50	300	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
17	310	127	330		+(*b*)	+(*b*)	−	−	+
18									
19	650	187	400		+(*b*) (*c*)	+(*b*) (*c*)	−	+	+
20	240	23	110	+(*c*)		+(*a*) (*c*)	+	−	+
21 *	1,300	895	850			+(*b*) (*c*)	−	+	+
22	3,800	790	1,110	+(*c*)	+(*b*) (*c*)	+(*b*) (*c*)	+	+	+
23 ‡	2,500	306	2,250	+(*c*)	+(*c*)	+(*c*)	+	+	+
24	3,900	350	1,400		+(*c*)	+(*b*) (*c*)	+	+	+
25									
26	700	190	680	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
27	470		340		+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
28	500		230			+(*c*)	−	+	+
29	360				+(*c*)	+(*c*)	−	+	+
30	320				+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
31	200	115	200		+(*c*)	+(*c*)	−	+	+
Average	2,957+	172+	4,487+						

*Dalecarlia reservoir by-passed from Aug. 21 to Dec. 31.

‡From this date samples taken from the inlet of Georgetown reservoir.

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

SEPTEMBER.

Date.	Number of bacteria per cubic centimeter			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Sept. 3	300	106	160	....	....	+(*c*)	—	—	+
4	600	208	250	....	+(*a*) (*c*)	+(*a*) (*c*)	—	+	+
5	360	....	550	....	....	+(*c*)	—	+	+
6	15,000	....	840	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
7	2,000	382	700	+(*c*)	+(*b*) (*c*)	+(*a*) (*b*)	+	+	+
8	....	....	....	....	....	....	....	....	....
9	220	300	....	....	+(*b*) (*c*)	+(*b*) (*c*)	—	+	+
10	18,000	530	....	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
11	2,700	340	800	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
12	1,000	716	800	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
13	2,300	510	860	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
14	2,400	566	500	+(*b*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
15	....	....	....	....	....	....	....	....	....
16	....	430	550	....	+(*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
17	420	....	1,250	....	+(*a*)	+(*a*) (*c*)	—	—	+
18	900	....	570	....	+(*c*)	+(*c*)	—	+	+
19	2,000	1,660	1,700	....	+(*b*) (*c*)	+(*b*) (*c*)	—	+	+
20	4,200	1,200	1,800	....	+(*b*) (*c*)	+(*b*) (*c*)	+	+	+
21	1,100	550	1,150	....	+(*c*)	+(*c*)	+	+	+
22	....	....	....	....	....	....	....	....	....
23	2,400	....	1,100	....	....	+(*c*)	+	+	+
24	4,000	....	1,300	....	+(*c*)	+(*c*)	+	+	+
25	56,000	4,000	1,080	+(*a*) (*b*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
26	13,000	2,625	5,000	+(*a*) (*b*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
27	4,000	850	4,300	+(*a*) (*b*) (*c*).	+(*a*) (*b*) (*c*)	+(*a*) (*c*)	+	+	+
28	15,000	865	4,200	+(*a*)	+(*a*) (*b*) (*c*)	+(*a*) (*c*)	+	+	+
29	....	....	....	....	....	....	....	....	....
30	....	3,500	12,800	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
Average	6,722+	590+	1,920+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

OCTOBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Oct. 1	600	550	6,000	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
2	4,400	285	3,200	+(*c*)	+(*b*) (*c*)	+(*b*) (*c*)	+	+	+
3	900	300	875	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
4	850	320	550	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
5	2,000	285	510	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
6	....	....	....	....	....	....	....	....	....
7	1,250	65	440	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
8	11,000	118	606	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
9	2,000	170	970	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	—	+	+
10	800	2,500	400	....	....	+(*b*) (*c*)	+	+	+
11	2,000	395	390	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*c*)	+	+	+
12	1,200	203	260	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
13	....	....	....	....	....	....	....	....	....
14	1,200	252	340	....	+(*c*)	+(*b*) (*c*)	—	+	+
15	900	....	660	....	+(*a*) (*c*)	+(*a*) (*c*)	—	+	+
16	....	290	3,550	....	+(*a*) (*b*)	+(*a*) (*b*) (*c*)	—	—	+
17	550	150	510	....	....	+(*a*) ((*b*) (*c*)	—	—	+
18	260	204	790	....	+(*b*) (*c*)	+(*b*) (*c*)	—	+	+
19	750	280	730	....	+(*c*)	+(*c*)	—	+	+
20	....	....	....	....	....	....	....	....	....
21	480	180	1,050	....	+(*c*)	+(*b*) (*c*)	—	+	+
22	230	108	850	....	....	....	+	+	+
23	250	190	480	....	....	....	—	—	+
24	300	80	1,400	....	+(*b*)	+(*c*)	—	—	+
25	450	50	480	....	....	+(*b*) (*c*)	—	—	+
26	450	74	350	....	....	+(*a*) (*c*)	—	+	+
27	....	....	....	....	....	....	....	....	....
28	190	202	550	....	....	....	—	—	—
29	380	320	1,325	....	+(*c*)	+(*c*)	—	+	+
30	450	100	500	....	....	+(*b*)	—	—	+
31	2,300	1,100	2,800	....	+(*c*)	+(*a*) (*b*) (*c*)	—	+	+
Average	1,390	337+	1,132+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

NOVEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Nov. 1..........	550	750	3,775	....	+(*c*).......	+(*a*) (*c*)...	−	+	+
2..........	900	146	800	....	....	+(*a*) (*c*)...	−	−	+
3..........	....	....	....	....	....	....	....	....	....
4..........	5,500	10,000	19,000	+(*a*) (*c*).	+(*a*) (*c*)...	+(*a*) (*c*)...	+	+	+
5..........	6,000	40,000	20,000	+(*a*) (*c*).	+(*a*)(*b*)(*c*).	+(*a*)(*b*)(*c*)	+	+	+
6..........	5,000	13,000	14,700	+(*c*).....	+(*a*)(*b*)(*c*).	+(*a*) (*c*)...	+	+	+
7..........	14,000	5,000	56,000	....	+(*a*) (*c*)...	+(*a*) (*c*)...	−	+	+
8..........	1,900	4,500	12,300	+(*a*)	+(*a*) (*c*)...	+(*a*) (*c*)...	−	+	+
9..........	4,000	6,000	7,900	+(*a*) (*c*).	+(*a*) (*c*)...	+(*a*) (*c*)...	+	+	+
10..........	....	....	....	....	....	....	....	....	....
11..........	1,200	1,100	7,200	....	....	+(*c*).......	−	+	+
12..........	7,500	1,850	2,600	....	+(*a*) (*c*)...	+(*a*) (*c*) ..	−	+	+
13..........	1,600	1,140	3,100	....	+(*a*)	+(*a*) (*c*) ..	−	+	+
14..........	2,700	1,080	2,650	....	+(*c*).......	+(*a*)(*b*)(*c*).	+	+	+
15..........	1,800	800	2,100	....	....	+(*a*) (*c*) ..	−	+	+
16..........	1,100	....	1,900	+(*c*).....	+(*c*).......	+(*c*).......	+	+	+
17..........	....	....	....	....	....	....	....	....	....
18..........	1,600	1,400	2,250	+(*c*).....	+(*c*).......	+(*c*).......	+	+	+
19..........	1,300	1,500	1,700	....	+(*c*).......	....	−	+	−
20..........	500	....	11,800	+(*c*).....	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
21..........	9,900	....	18,800	+(*c*).....	+(*a*) (*c*) .	+(*a*) (*c*) ..	+	+	+
22..........	10,000	....	13,700	+(*c*).....	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
23..........	18,000	....	24,500	+(*a*)	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
24..........	....	....	....	....	....	....	....	....	....
25..........	50,000	....	14,200	+(*a*) (*c*).	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
26..........	40,000	....	36,000	+(*c*).....	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
27..........	16,000	....	74,000	+(*c*).....	+(*a*) (*c*) ..	+(*a*) (*c*) ..	+	+	+
28..........	....	....	....	....	....	....	....	....	....
29..........	10,000	....	139,600	....	+(*a*) (*c*) ..	+(*a*) (*c*) ..	−	+	+
30..........	3,800	....	10,800	....	....	+(*c*)	−	+	+
Average.....	9,634	5,885+	20,055−						

THE FILTERED RIVER WATER.

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir.

[Daily examinations made by the (*a*) Laboratory of the filtration plant; (*b*) Bureau of Plant Industry of Agricultural Department; (*c*) hygienic laboratory, Public Health and Marine-Hospital Service.]

MAY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
May 15	21	3	24					—	
16	13	12	29					—	
17	35	10	33					—	
18	16	3	22					—	
19									
20	24	16	25					—	
21	27	13	28					—	
22	28	6	29					—	
23	20	9	24					—	
24	20	15	28					—	
25	39	12	20					—	
26									
27	25		21					—	
28	39	13	14					—	
29	35		16					—	
30									
31	32	5	14			+ (*c*)		—	+
Average	26+	9+	23+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

JUNE.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
June 1	24	5	(?)	....	....	....	....	—	....
2	....	....	....	....	....	....	....	....	....
3	28	4	18	....	....	....	....	—	....
4	23	9	16	....	....	....	....	—	....
5	24	7	11	....	....	....	....	—	....
6	10	14	32	....	....	....	....	—	....
7	13	30	18	....	....	....	....	—	....
8	22	11	(?)	....	....	....	....	—	....
9	....	....	....	....	....	....	....	—	....
10	16	20	30	....	....	....	....	—	....
11	21	13	70	....	....	....	....	—	....
12	16	14	39	....	....	....	....	—	....
13	24	300	35	....	....	....	....	—	+
14	34	17	25	....	....	....	....	—	....
15	10	400	27	....	....	....	....	—	....
16	....	....	....	....	....	....	....	....	....
17	30	16	35	....	....	....	....	—	....
18	21	19	38	....	....	....	....	—	....
19	20	15	23	....	....	....	....	—	....
20	9	9	(?)	....	....	....	....	—	....
21	17	21	22	....	....	....	....	—	....
22	8	19	22	....	....	....	....	—	....
23	....	....	....	....	....	....	....	....	....
24	19	11	26	....	....	....	....	—	....
25	10	14	26	....	....	....	....	—	....
26	21	30	37	....	....	....	....	—	....
27	13	10	54	....	....	....	....	—	+
28	11	134	(?)	....	....	....	....	—	....
29	13	4	22	....	....	....	....	—	....
30	....	....	....	....	....	....	....	....	....
Average	18+	45+	29+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

JULY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
July 1	14	17	27	......	......	......	......	–	......
2	11	30	33	......	......	......	......	–	......
3	......	23	36	......	......	......	......	–	......
4	22	......	......	......	......	......	......	......	......
5	32	11	34	......	......	......	......	–	......
6	33	8	16	......	......	......	......	–	......
7	......	......	......	......	......	......	......	......	......
8	20	12	54	......	......	......	......	–	......
9	8	3	32	......	......	......	......	–	......
10	......	90	21	......	......	......	......	–	......
11	7	13	36	......	......	......	......	–	......
12	7	16	22	......	......	......	......	–	+
13	12	13	24	......	......	......	......	–	......
14	12	......	......	......	......	......	......	......	......
15	21	......	34	......	......	......	......	–	......
16	19	100	20	......	......	......	......	–	......
17	6	17	70	......	......	......	......	–	......
18	9	13	49	......	......	......	......	–	+
19	19	22	28	......	......	......	......	–	......
20	......	8	41	......	......	......	......	–	......
21	......	......	......	......	......	......	......	......	......
22	21	46	29	......	......	......	......	–	......
23	25	......	30	......	......	......	......	–	......
24	20	7	33	......	......	......	......	–	......
25	14	63	35	......	......	......	......	–	......
26	11	15	24	......	......	......	......	–	......
27	17	16	24	......	......	......	......	–	......
28	18	......	......	......	......	......	......	......	......
29	23	2	34	......	......	......	......	–	......
30	......	6	34	......	......	......	......	–	......
31	......	15	114	......	......	......	......	–	......
Average	16+	24+	36+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

AUGUST.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Aug. 1	9	10	28	....	....	....	....	—	....
2	6	37	28	....	....	....	....	—	....
3	18	2	....	....	....	....	....	....	....
4	20	....	....	....	....	....	....	....	....
5	15	5	26	....	....	....	....	—	....
6	19	3	22	....	....	....	....	—	....
7	27	7	(?)	....	....	....	....	—	....
8	8	16	19	....	....	....	....	—	....
9	8	8	46	....	....	....	....	—	....
10	9	....	36	....	....	....	....	—	....
11	17	....	....	....	....	....	....	....	....
12	10	2	34	....	....	+(*c*)	....	—	+
13	7	11	47	....	....	....	....	—	....
14	14	17	54	....	....	....	....	—	....
15	10	20	44	....	....	....	....	—	....
16	10	15	30	....	....	....	....	—	....
17	24	74	40	....	....	....	....	—	....
18	32	....	....	....	....	....	....	....	....
19	21	15	32	....	....	....	....	—	....
20	27	12	34	....	....	....	....	—	+
21[a]	21	21	39	....	....	....	....	—	+
22	20	3	35	....	....	....	....	—	....
23	18	22	45	....	....	....	....	—	....
24	34	15	50	....	....	....	....	—	....
25	22	....	....	....	....	....	....	....	....
26	25	13	35	....	....	....	....	—	....
27	10	....	32	....	....	....	....	—	....
28	5	....	26	....	....	....	....	—	....
29	9	....	(?)	....	....	....	....	—	....
30	25	....	(?)	....	....	....	....	—	....
31	12	....	46	....	....	....	....	—	....
Average	16+	15+	34+						

[a] Dalecarlia Reservoir by-passed from Aug. 21.

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

SEPTEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Sept. 1	65								
2	39								
3	36	22						–	
4	23	7	52					–	
5	51		65			+ (*c*)		–	+
6	38		54					–	+
7	34	5	90					–	
8	33								
9	30	7	(?)					–	
10	30	6	80					–	+
11	20	6	30					–	
12	25	5	30					–	+
13	62	13	58			+(*a*)		–	
14	42	5	23					–	
15	70								
16	23	6	100					–	
17	10		26					–	
18	9		31					–	
19	6	12	23					–	+
20		11	41					–	
21	19	7	22					–	
22	20								
23	18		140					–	
24	190		70					–	+
25	24	2	48					–	
26	47	1	190					–	
27	19	8	180			+ (*c*)		–	+
28	24	500	190			+ (*c*)		+	+
29	55								
30		10,000	220		+ (*b*) (*c*)	+ (*b*) (*c*)		+	+
Average	37+	590+	80+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

OCTOBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Oct. 1	42	12	260	....	....	....	....	+	+
2	220	45	240	....	+ (*b*) (*c*)..	+ (*b*) (*c*)..	....	+	+
3	31	10	56	....	....	+ (*b*).....	....	—	+
4	38	14	56	....	....	+(*a*)(*b*)(*c*)	....	+	+
5	44	23	22	....	....	+ (*b*) (*c*)..	....	—	+
6	30	....	....	....	....	+ (*a*).....	....	....	....
7	40	3	34	....	....	+ (*b*) (*c*)..	....	—	+
8	24	4	28	....	....	+ (*b*) (*c*)..	....	—	+
9	30	3	70	....	....	+ (*b*).....	....	—	—
10	26	3	18	....	....	....	....	—	—
11	44	4	110	....		....	....	—	—
12	75	....	100	....	....	+ (*c*).....	....	—	+
13	38	....	....	....	....	....	....	....	....
14	43	10	28	....	....	....	....	—	....
15	50	....	40	....	....	....	....	—	....
16	13	9	20	....	....	....	....	—	....
17	14	10	100	....	....	....	....	—	....
18	19	9	18	....	....	....	....	—	+
19	21	6	16	....	....	+ (*c*).....	....	—	+
20	32	....	....	....	....	....	....	....	....
21	28	5	60	....	....	....	....	—	—
22	13	4	39	....	....	....	....	—	—
23	11	....	180	....	....	....	....	—	—
24	14	6	500	....	....	....	....	—	—
25	19	6	140	....	....	....	....	—	—
26	18	9	20	....	....	....	....	—	—
27	....	....	....	....	....	....	....	....	....
28	15	6	30	....	....	....	....	—	—
29	10	7	30	....	....	....	....	—	....
30	4	2	70	....	....	....	....	—	....
31	8	9	18	....	....	....	....	—	....
Average	41+	9+	84+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

NOVEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Nov. 1	18	27	130	....	....	....	....	−	....
2	6	7	21	....	....	....	....	−	....
3	17	....	....	....	....	....	....	....	....
4	12	7	21	....	....	+ (*c*)	....	−	+
5	10	6	15	....	....	....	....	−	....
6	12	6	17	....	....	....	....	−	....
7	....	5	59	....	....	....	....	−	+
8	17	10	30	....	....	....	....	−	....
9	21	8	14	....	....	....	....	−	....
10	25	....	....	....	....	....	....	....	....
11	23	3	14	....	....	....	....	−	....
12	22	8	19	....	....	....	....	−	....
13	17	4	33	....	....	....	....	−	....
14	10	8	30	....	....	....	....	−	....
15	17	6	100	....	....	....	....	−	+
16	28	....	68	....	....	....	....	+	+
17	20	....	....	....	....	....	....	....	....
18	17	11	47	....	....	+ (*c*)	....	−	+
19	16	12	98	....	....	....	....	−	−
20	12	....	33	....	....	....	....	−	−
21	15	....	25	....	....	....	....	−	−
22	20	....	44	....	....	....	....	−	+
23	21	....	36	....	....	+ (*c*)	....	−	+
24	55	....	....	....	....	....	....	....	....
25	48	....	66	....	....	....	....	+	−
26	43	....	155	....	....	....	....	−	−
27	60	....	103	....	....	+ (*c*)	....	+	+
28	70	....	....	....	....	....	....	....	....
29	70	....	185	....	....	....	....	−	+
30	60	....	216	....	....	....	....	−	+
Average	26+	8+	63+						

These results show the great extent of bacterial purification which the water underwent in its passage through the storage reservoirs and filter beds.

The effluent from the filter beds during the period extending from May 15 to August 31 can not be regarded from a bacteriological standpoint other than as a water of good sanitary quality. During this period, of the 84 samples of which counts were made by us, the average number of bacteria per cubic centimeter was about 31. Ninety-one samples were examined in quantities of 0.1 c. c., 1 c. c., and 10 c. c., for the presence of the colon bacillus. In none of the 0.1 c. c. and 1 c. c. quantities was *B. coli* found, and of the 10 c. c. quantities, *B. coli* was found in only 2, or about 2.2 per cent.

In 1906 the samples of water, taken at intervals of a few days during the period from July 25 to August 31, from the filtered water reservoir, and examined by us, showed an average of 44 bacteria per cubic centimeter. Of 13 samples examined in this period, *B. coli* was found in 1 c. c. once and in 10 c. c. twice. Unfortunately the number of samples from the filtered water reservoir examined by us in 1906 was too small to compare aptly with the results of 1907, but during the period extending from July 16 to August 31, 1906, we examined 60 samples of tap water taken from taps in various parts of the city. During this period the average number of bacteria per cubic centimeter in the tap water was about 67, and of the 60 samples, *B. coli* was found in quantities of 1 c. c. twice, and in quantities of 10 c. c. sixteen times, or in about 3.3 per cent of the 1 c. c. samples and in about 26.6 per cent of the 10 c. c. samples.

It will be observed in the above tables that the remarkably high degree of purity of the water accomplished by the storage and filtration in the summer of 1907 was not maintained during September and October. According to our own results, the total bacterial content of the filtered water during September averaged 80 per cubic centimeter, and during October, 84 per cubic centimeter. In September, of the 1 c. c. samples examined, *B. coli* was found in 4.3 per cent and of the 10 c. c. samples in 17.4 per cent. In October, *B. coli* was found in 3.6 per cent of the 1 c. c. samples and in 26 per cent of the 10 c. c. samples examined. In this connection it is interesting to note that Dalecarlia, the principal sedimentation reservoir, was by-passed from August 21 to December 31, 1907.

It should be stated that at present the generally accepted view is that the colon bacillus is only an index of pollution. It suggests danger, but not the amount of danger. In other words, a water may contain a large number of colon bacilli and yet be entirely free of typhoid bacilli.

In November the average bacterial content of the filtered water was 63 per cubic centimeter, and *B. coli* was found in 16 per cent of the 10 c. c. samples examined. The low bacterial content of the filtered water in November is interesting when considered in connection with the fact that the raw river water during this month averaged (our results) over 15,000 bacteria per cubic centimeter more than it did in either September or October.

According to bacteriological standards of pure water, the water delivered through the filtration plant to the city of Washington during May, June, July, and August of 1907 was of a high degree of purity, and on this evidence alone it would appear justifiable to exclude this water as a factor in the conveyance of typhoid fever infection.

The filtered water this summer was of a better quality than it was in the summer of 1906; that is, as nearly as we can judge by our bacteriological results of this summer in comparison with those of our more limited studies in 1906. The improvement in the water being followed by the lower rate of prevalence of typhoid fever in the summer of 1907 suggests cause and effect. The known factors, other than water, seemed, so far as the results of our epidemiological studies indicate, to have operated relatively to even a less extent during the summer of 1906 than in that of 1907. This suggests that the difference in morbidity rate of typhoid fever for the two summers was due to a difference in the amount of the water-borne typhoid fever infection.

However, it should be borne in mind that the prevalence of typhoid fever in Washington and other communities has varied considerably in different years during which the conditions as to water, etc., have, so far as known, remained approximately the same.

Further, it should be stated that the improvement in the quality of the Potomac River water accomplished by sand filtration in 1906 was not followed by a lowering of the typhoid fever rate. In cities where infected water is known to have played a conspicuous rôle, the rule has been that the filtration or improvement of the water supply has been followed by an immediate impress upon the height of the typhoid curve. In Paterson, N. J., however, the decline in the rate was not apparent until the second year following the filtration of the public water supply. At Lawrence, Mass., the filtration of the city water supply was followed the first year by a marked decrease, but it required three years to produce its maximum effect. There has been much discussion in regard to the fact that in 1906 the improvement in the Potomac River water by filtration was not followed by an apparent lowering of the typhoid fever rate in Washington. The view is held by some that this fact strongly indicates that little if any of the typhoid fever in Washington was ever conveyed in the Potomac water. In this connection it is interesting to note that according to our results and those obtained in the laboratory at the filtration plant in 1906. from 55 to 86 per cent of the total bacteria in the Potomac River water are removed by storage in the three sedimentation reservoirs before the water is applied to the filter beds. These storage reservoirs have been in operation since 1902. During the two or three years prior to the installation of the filters no systematic bacteriologic examination of the water was made; so we do not know what was the bacterial content of the water supplied to Washington during that period and can not accurately compare it with that of the water after filtration was begun. We have no accurate data on which to base a comparison of the amount of typhoid fever infected excreta poured into the Potomac River in 1906 with that of previous years. We know practically nothing of the condition of symbiosis, etc., under which the typhoid bacillus will live best in a river water during certain seasons and perhaps certain years.

The quite regular seasonal (summer) prevalence of typhoid fever in Washington, together with the absence of frequent winter and spring outbreaks, is regarded by some as good evidence that the Potomac River water has played but a minor rôle in transmitting the infection. In Albany and other cities where contaminated water is known to have played a prominent rôle, improvement in the water supply not only diminished the total amount of typhoid fever, but changed the character of the typhoid curve. The rule is that where there is much water-borne typhoid there is also comparatively much winter and spring typhoid. In Albany the filtration of the water markedly reduced the prevalence of typhoid fever throughout the entire year, but most markedly during the winter and spring, during which seasons there was, prior to filtration, relatively much more typhoid than during the summer season. On the other hand, Washington has had relatively little winter and spring typhoid; the annual curve, even when excessively high and before any attempted purification of the water supply, shows a marked summer maximum or the so-called "normal" curve. The Washington curve has changed markedly in height, but little in character.

In the majority of cities supplied with a polluted water and having a high typhoid rate in the winter and spring, the pollution of the water has been nearer the intake and presumably much more concentrated than is the case of Washington's water supply. The vast bulk of the pollution of the Potomac occurs at points over 100 miles above the intake of the water supply for Washington, and little or practically no direct pollution takes place within 19 miles of the intake. Therefore, as the dilution of the sewage must be tremendous by the time it reaches the intake at Great Falls it may be that the small amount of typhoid infection finding its way into the river from the relatively small number of cases occurring on the watershed during the winter and early spring is not sufficient to affect markedly the prevalence of the disease in Washington, but with the increase of cases on the watershed in the summer there may be, in spite of the dilution, enough typhoid infection in the water to occasion a marked increase in the prevalence of the disease in Washington. In this connection reference should be made to Kober's observations on the effects of the typhoid epidemic of 1889–1890 at Cumberland, Md., upon the prevalence of the disease in Washington.[a]

We have noted that in dry and warm seasons the present system of storage and filtration of the Potomac River water will give a water of good sanitary quality, but in periods of high turbidity, especially in winter, fine silt in the river water does not settle so well, nor is it all retained by the sand filters. With the increased amount of this silt in the effluent is an increased number of bacteria.

MILK.

The 523 cases considered as having contracted the infection in the District of Columbia gave the following history as to the use of milk during the thirty days prior to illness:

As a beverage	290
On fruits or cereals, but not as a beverage	118
In hot tea or coffee only	44
As ice cream only	28
In no way	38
Not determined	5
Total	523

Of the cases using milk as a beverage or on fruits and cereals 6 used boiled milk exclusively.

One distinct milk outbreak occurred. This was among the customers of milk dealer No. 163. There were in the course of this outbreak 31 cases with the onset of illness between June 8 and August 17. (See Chart No. 4.) Of these cases 2 gave a history of having used boiled milk only during the thirty days prior to onset, and for one other infection by contact seemed more probable. Eliminating these 3 cases there are left 28, considered as being quite definitely attributable to milk infection.

The evidence that these cases were due to milk infection was not absolutely positive, but it seemed sufficiently strong to justify attributing these cases to milk. Thus, there was a sudden and marked increase in the number of cases among the customers of this dealer. The number of cases among his customers was far in excess of the rate of prevalence among persons living in the same neighborhoods and under practically similar conditions but obtaining milk from other sources.

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[a] Report on the pollution of rivers, by Henry Talbott, Chairman of Committee on River Pollution, to the Game and Fish Protection Association of the District of Columbia. Government Printing Office, Washington, 1898, p. 32.

There were several instances of two or more cases in the same family with onsets on the same day. About 200 families obtained milk from this dairy, and estimating the number of persons in a family at 5, there were about 1,000 persons exposed to this milk infection. The histories obtained as to the ways in which milk was used by the 28 cases during the thirty days prior to the onset of illness were as follows:

	Cases.
As a beverage	20
On fruits and cereals, but not as a beverage.	6
In tea or coffee only	2
Total	28

The ages of the cases were as follows:

Years:	Cases.
0–4	4
5–9	5
10–14	2
15–19	3
20–24	7
25–29	4
30–34	2
40–44	1
Total	28

There were six instances in which the person affected was the only member of the family who used milk freely as a beverage, the other members using it chiefly in fruits, cereals, and coffee. On the other hand, and showing how misleading the exceptional case may be, there was one instance of three children in a family all of whom used milk as a beverage, but the child who drank milk in smallest quantity was the one affected.

In another family there were three children, aged about 2, 4, and 6 years, all of whom used milk as a beverage. For the youngest child the milk was sterilized. This child remained well, while the two who drank the raw milk had typhoid fever, being stricken on about the same date.

The dairy was supplied with milk by two dairy farms. At the beginning of the outbreak one of these farms was inspected by representatives of the health office. No typhoid fever cases were found on this farm; but on account of general conditions the sale of milk from it in the District of Columbia was discontinued by order of the health officer on July 10.

The outbreak continued after an interval more than covering the usual incubation period of typhoid fever (in cases of milk infection) had elapsed after the discontinuance of milk from this farm. So it seems that the bulk of the infection did not come in the milk from this farm.

The inspection of the dairy showed nothing immediate to account for the infection. Three of the men who worked at the dairy gave a history of having had typhoid fever within four to twenty years previous. Specimens of their stools and urine were examined at the Hygienic Laboratory, but all were negative for *B. typhosus.*

The sale of milk from this dairy was discontinued by order of the health officer on August 6.

One of us, conjointly with a representative of the health office, made an inspection of the other farm supplying this dairyman with milk, and the evidence obtained indicated quite strongly that this farm was the source of the infection for the majority of the cases. The owner of this farm did most of the handling of the milk. He was taken ill about July 1 with diarrhea and fever and confined to bed for a day or two. For about five weeks from that time he was complaining, having to lie down for an hour or two most every day; had slight fever, which he thought was malarial. He was engaged in handling the milk nearly every day throughout his illness.

In the neighborhood of this dairy farm were seven families which obtained milk from the farm. In one of these families a child was taken ill about June 8 and continued ill with fever for about four weeks. Milk bottles from the dairy farm were delivered to and collected from this house daily throughout the child's illness.

In another of these families were three cases, clinically typical cases of typhoid fever, having the onset of illness in the early part of August.

These five cases all occurred in the State of Maryland and are not included in our figures.

The sale of milk from this farm was discontinued by order of the health officer on August 14.

Besides this pronounced outbreak there were suggestive groups of cases on the routes of milk dealers as follows (see Chart No. 4):

	Cases.
Dealer No. 8: Onset of cases from August 5 to September 7	15
Dealer No. 46: Onset of cases from August 14 to September 4	13
Dealer No. 164: Onset of cases from August 27 to September 5	4
Dealer No. 44: Onset of cases from August 9 to 26	8
Total	40

No positive evidence could be obtained that these cases were due to milk infection, yet the grouping of the cases, etc., suggest so strongly that these were small milk outbreaks that it seems fair to estimate the chances of these 40 cases as having been due to milk infection at about 50 per cent; and so, adding 20 of these cases to the 28 quite definitely attributable to milk infection, we have 48, or about 7 per

cent of the 670 cases, or 9 per cent of the 523 cases, attributable to milk infection.

Attention is called to the difference for the two years in the ratios of number of typhoid cases to amount of milk sold of the two large milk dealers who had outbreaks of typhoid fever among their customers in 1906 but not in 1907 (see Chart No. 3 of Hygienic Laboratory Bulletin 35 and Chart No. 4 of this bulletin), thus:

Number of cases per 100,000 gallons of milk sold.

	1906.	1907.
Milk dealer No. 4	52.5	21.6
Milk dealer No. 13	113.9	6.5

Of the 866 cases investigated by us in 1906, about 10 per cent were attributed to infection conveyed by milk in the course of pronounced outbreaks among the customers of three milk dealers.

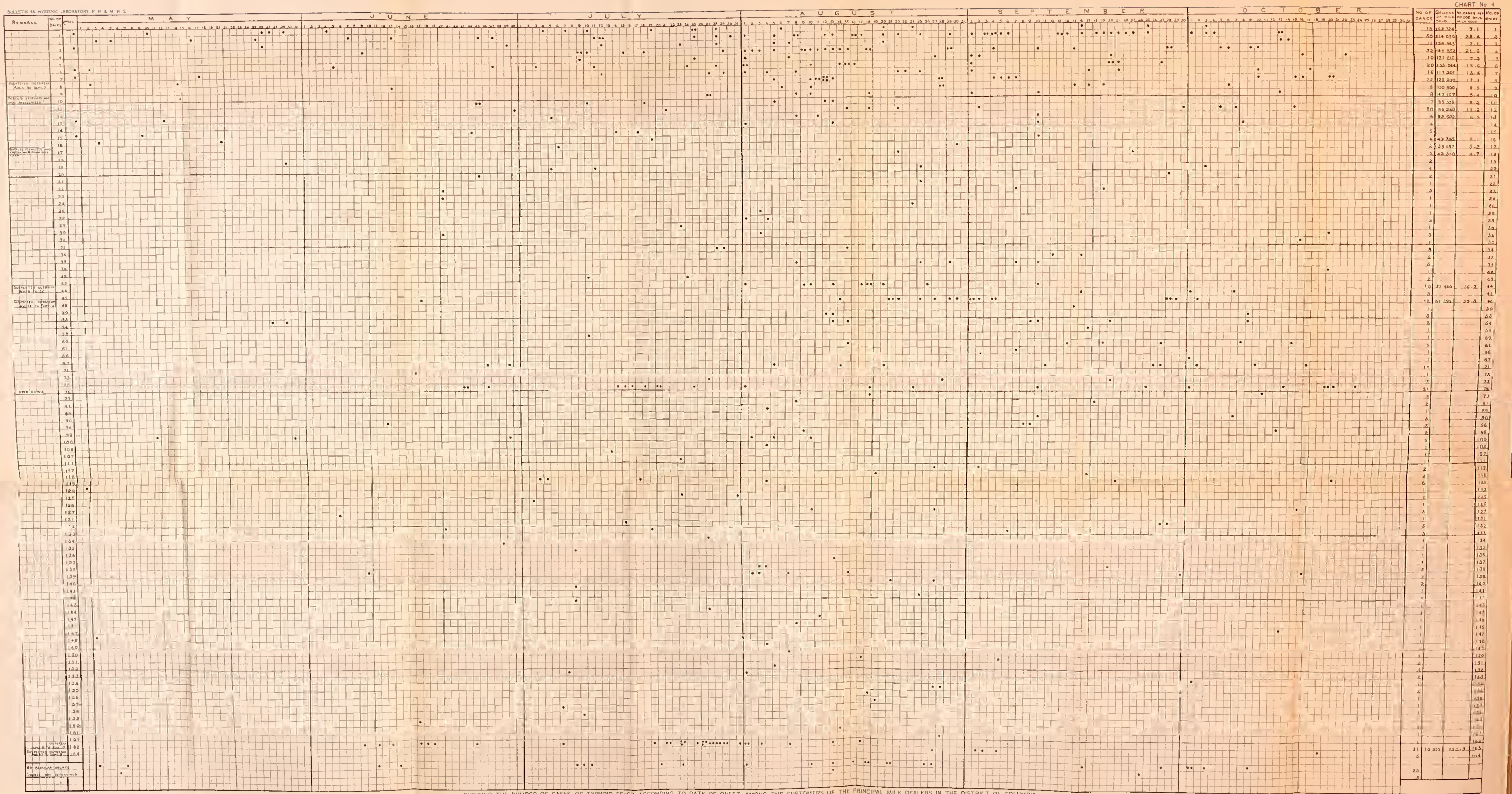
Besides these cases, quite definitely attributable to infected milk, there no doubt were, during both years, scattering cases due to, but which could not be traced to, milk. The number of such cases is of course problematical.

The large proportion of cases of typhoid fever in families of the better class and the conspicuously large proportion of cases among children in Washington suggest that milk plays a larger part in the spread of the infection than we have been able to definitely determine by epidemiological studies. A study of Chart No. 3 of Hygienic Laboratory Bulletin 35 and the corresponding chart (No. 4) of this bulletin seems to support such a view. In this connection it is interesting to note that for both 1906 and 1907 milk dealer No. 10 had a conspicuously small number of cases of typhoid fever proportionate to the amount of milk sold. This dealer is the only one in Washington who both sterilizes the bottles and pasteurizes the milk. The low typhoid fever rate among the customers of milk dealer No. 10 is significant, and perhaps is a fair index of the result which would be accomplished by the pasteurization of the general milk supply of Washington.

Considering only the cases definitely attributable to milk and the number of secondary cases contracting the infection directly or indirectly from them, it is evident that the milk problem in the District of Columbia is grave and important, and one requiring the serious attention of sanitary authorities.

The milk supply of Washington is obtained from about 1,000 dairy farms scattered through Virginia, Maryland, the District of Columbia, and a few even in Pennsylvania and New York. Some of this milk is sent directly from the dairy farms to the consumer; the bulk

CHART No. 4



SHOWING THE NUMBER OF CASES OF TYPHOID FEVER, ACCORDING TO DATE OF ONSET, AMONG THE CUSTOMERS OF THE PRINCIPAL MILK DEALERS IN THE DISTRICT OF COLUMBIA

THE NUMBER OF THE DAIRY CORRESPONDS TO THE NUMBER OF THE DAIRY ON CHART No 3, BULLETIN No 35, OF THE HYGIENIC LABORATORY, P H & M H S.

NOTE THE RELATIVELY SMALL NUMBER OF CASES PER 100,000 GALLONS OF MILK SOLD FOR DAIRYMAN No 10 ON THIS CHART AND ON CHART No 3 OF BULLETIN No 35, HYGIENIC LABORATORY, P H & M H S. THIS DAIRY IS THE ONLY ONE IN WASHINGTON THAT BOTH STERILIZES BOTTLES AND PASTEURIZES MILK

of it, however, is shipped from the farms to the 65 or 70 dairies operating in the District of Columbia, where it is mixed, bottled, etc., before delivery. Some of the dairies in Washington receive milk from as many as 30 to 40 farms. It is evident that a supervision over these various dairy farms and dairies sufficiently thorough to prevent the introduction of typhoid fever infection into milk would require a large force of inspectors and could be done only at a great cost. Therefore the treatment of milk before it is delivered to the consumer by some process (pasteurization), which will destroy any possible infection contained therein and at the same time not impair the food value of the milk, seems to be at the present time the cheapest and most practical method of preventing the conveyance of typhoid fever infection by milk.

There can be no more objection to the pasteurization of milk for the use of adults and of children over three years old than there is to the cooking of meat. The fresh clean "certified"[a] or "inspected"[a] milk for infant feeding and special uses needs no purification. Pasteurization may not be the ideal to be attained, but until we can be assured that the general market milk is free from danger the only protection is to destroy the infection by means of heat. Pasteurization not only protects the community against much of the typhoid fever, but also against some of the tuberculosis, scarlet fever, diphtheria, infant diarrheas, etc. For a full discussion of this subject see the article on Pasteurization in Hygienic Laboratory Bulletin No. 41, page 591.

ICE CREAM.

The history of the cases in regard to the use of ice cream within thirty days prior to the onset of illness was as follows: Three hundred and forty-five ate it from time to time, 163 ate none, and for 15 the facts were not determined. No cases this year were traced to infected ice cream, but in view of the bad sanitary conditions under which much of the ice cream sold in Washington is made and handled (particularly by street vendors) a certain number of scattering cases probably were caused by infection through ice cream.

RAW SHELLFISH.

The following table shows the history of the cases in regard to the eating of raw oysters and clams from time to time within thirty days prior to onset of illness:

Shellfish.	Yes.	No.	Not determined.
Oysters	23	488	12
Clams	17	494	12

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[a] For a definition of these terms see "The classification of market milk," by A. D. Melvin, Hygienic Laboratory Bulletin No. 41, p. 559.

The small proportion of cases giving a history of having eaten uncooked shellfish shows that shellfish this year, as was the case in 1906, could not have been the means of transmitting the infection to any considerable number of cases.

All but 9 of the 523 cases occurred between May 1 and November 1, during which period comparatively few raw oysters or clams are eaten in the District of Columbia.

OCCUPATION.

In the following table is given the occupation of the cases, along with the number of persons in the District of Columbia, according to the census of 1900, engaged in such occupations:

Occupations.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male.	Female.	Total.	Male.	Female.	Total.
Professional pursuits:						
Electricians	4	0	4	461	0	461
Journalists	1	0	1	398	0	398
Lawyers	5	0	5	1,445	0	1,445
Musicians and teachers of music	3	0	3	414	307	721
Officials (Government)	2	0	2	900	0	900
Physicians and surgeons	1	0	1	881	0	881
Domestic and personal service:						
Barbers, hairdressers	2	0	2	860	0	860
Bartenders	2	0	2	591	0	591
Housekeepers, stewardesses	0	1	1	0	529	529
Janitors, sextons	1	0	1	511	0	511
Laborers, not specified	17	0	17	12,476	263	12,739
Launderers, laundresses	2	1	3	474	7,192	7,666
Nurses, midwives	1	5	6	231	1,311	1,542
Restaurants, saloon keepers	3	0	3	612	112	724
Servants and waiters	2	16	18	2,898	15,231	20,129
Soldiers, sailors, marines (United States)	3	0	3	745	0	745
Watchmen, policemen, firemen, etc	2	0	2	1,667	0	1,667
Trade and transportation:						
Agents	1	0	1	1,511	0	1,511
Bookkeepers, accountants	1	2	3	837	482	1,319
Clerks, copyists	21	9	30	11,523	4,697	14,220
Draymen, hackmen, teamsters	16	0	16	3,994	0	3,994
Hostlers	5	0	5	410	0	410
Hucksters, peddlers	1	0	1	526	0	526
Merchants, dealers (retail)	2	1	3	3,945	418	4.363
Messengers, errand and office boys	5	0	5	1,345	0	1,345
Porters, helpers in stores	6	0	6	921	0	931
Salesmen, saleswomen	5	2	7	2,644	1,320	3,964
Steam railroad employees	7	1	8	1,185	(?)	1,185
Stenographers and typewriters	0	5	5	521	708	1.229
Street railway employees	7	0	7	817	0	817
Telegraph and telephone operators	2	0	2	307	(?)	307
Mechanical and manufacturing:						
Bakers	0	1	1	622	?	625
Blacksmiths	1	0	1	775	0	772
Milliners	0	1	1	0	344	344

Occupations.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male.	Female.	Total.	Male.	Female.	Total.
Mechanical and manufacturing—Continued.						
Boot and shoemakers, repairers	2	0	2	496	0	496
Carpenters, joiners	7	0	7	2,298	0	2,298
Dressmakers	0	2	2	0	2,993	2,993
Engineers, firemen (not locomotive)	4	0	4	1,116	0	1,116
Iron and steel workers	4	0	4	300	0	300
Manufacturers, officials, etc	1	0	1	925	0	925
Machinists	13	0	13	1,392	0	1,392
Masons, brick and stone	3	0	3	1,153	0	1,153
Paper hangers	2	0	2	363	0	363
Plasterers	1	0	1	465	0	465
Plumbers, gas and steam fitters	5	0	5	1,074	0	1,074
Printers, lithographers, pressmen	5	4	9	2,942	481	3,323
Tailors	1	0	1	616	0	616
Miscellaneous:						
Persons attending school	84	85	169	18,788	21,116	39,904
Infants and small children	25	20	45	(?)	(?)	(?)
Housewives	0	48	48	0	35,000	[a] 35,000
Postmen	2	0	2			
Inmates of institutions	3	1	4			
No occupation	1	12	13			
Tinners and stove fitters	1	0	1			
Coppersmiths	1	0	1			
Inspector, city water department	1	0	1			
Elevator boys	2	0	2			
Dyers, cleaners	1	0	1			
Prostitutes	0	1	1			
Pharmacists	1	0	1			
Chaffeurs	1	0	1			
Veterinary surgeons	1	0	1			
Manicurists	0	1	1			
Not determined	1	0	1			
Total	304	219	523			

[a] Our estimate.

It is evident that the disease was fairly uniformly distributed among persons regardless of occupation. The rate is seen to have been somewhat disproportionately high among electricians, hostlers, porters and helpers in stores, steam railroad employees, street railway employees, telegraph and telephone operators. iron and steel workers, machinists, paper hangers and plumbers.

INFECTION BY CONTACT.

Of the 523 cases, 70, or about 13 per cent, gave a history of having had within thirty days prior to onset of illness free and intimate association with typhoid patients in the febrile stage of the disease, and were attributable to infection by direct contact. In these 70 cases are not included several giving a history of contact, but considered as being more probably attributable to some other factor such as milk.

The following table gives the interval in days elapsing between the definite onset of illness of the 70 secondary cases and the definite onset of the primary cases from which these 70 cases were considered to have contracted the infection:

Number of days elapsing between onset of primary and secondary case.	Number of secondary cases.	Number of days elapsing between onset of primary and secondary case.	Number of secondary cases.
7	2	35	1
8	3	36	1
9	4	39	1
10	3	40	1
11	3	41	1
12	2	44	1
14	1	46	1
16	1	48	1
17	1	51	1
18	2	53	1
19	2	54	1
20	1	Not accurately determined	6
21	1	Interval not determined, secondary cases being among physicians, nurses, helpers, or inmates of hospitals who were more or less constantly exposed	12
22	1		
24	2		
25	3		
27	1		
28	2		
29	2		
30	2	Total	70
31	1		
33	1		

These figures emphasize the importance of exercising the utmost precautions to prevent the spread of infection from typhoid fever patients from the onset to the end of the illness.

Among the cases not definitely attributable to a known factor there were 16 which gave a history of association with febrile cases suspected to be but not reported as typhoid fever. In 45 cases indirect conveyance of infection by persons or flies was strongly suggested. In determining that conveyance of infection by flies was probable the proximity of the house occupied by the primary case to that occupied by the secondary case, the number of flies[a] in the neighborhood and the ease with which they could pass from the infected excreta of the primary case to the food of the secondary case, and all other conditions thought to have any bearing on the situation, were taken into consideration.

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[a] It is difficult to estimate the part played by flies in spreading the infection of typhoid in a city having a good sewerage system. However, it is evidently advisable to lessen their number, which may be successfully done in a community by the proper disposal of manure.

Four cases gave a history of association with persons convalescing from typhoid fever.

It seems fair to estimate the chances of these 65 cases having become infected by contact at about 50 per cent; and so, adding 32 of these to the 70 considered as almost certainly having been infected by contact, there were 102, or about 19 per cent, of the 523 cases attributable to contact. These 102 cases compose about 15 per cent of the total 670 cases investigated, as compared with 6.23 per cent (estimated on the same basis of probability) of the 866 cases investigated in 1906.

In endeavoring to determine what part, if any, chronic bacillus carriers have in the dissemination of typhoid fever infection in the District of Columbia, the following data in regard to association of the 523 cases with persons who had had typhoid fever within the five years previous were obtained:

Association 30 days prior to onset with persons who had had typhoid fever within—	Amount of association.			
	Intimate.	Fairly intimate.	Slight.	Total cases.
Six months	2	1	1	4
One year	8	5	3	16
Two years	3	5	2	10
Three years	7	5	2	14
Four years	2	2	0	4
Five years	6	3	2	11
None known				464
Total	28	21	10	523

For people living in large cities, exact data as to association during a period of thirty days with persons who have had, sometime within the previous five years, an attack of typhoid fever would be almost if not quite impossible to obtain, and so the data presented in the above table refer almost entirely to association with other members of the family or with intimate friends. The number of cases giving a history of such association is, therefore, necessarily far below the actual.

Effort was made to obtain from persons who had had typhoid within five years and with whom the cases had associated specimens of feces for bacteriological examination; but this effort did not meet with much success, as many of the people showed a reluctance, from perhaps some peculiar sense of delicacy, toward collection of the specimens. Some of the specimens were too old before they reached the laboratory to give reliable results.

Data relative to newcomers in the residences at which cases occurred are given in the following table. By newcomers we mean persons who moved into a household within three months of the onset of the case.

Number of cases at private residences at which one or more newcomers lived within thirty days prior to occurrence of case	36
At hotels, hospitals, and other public institutions	21
Not determined	3
No newcomers	463
Total	523

Of the newcomers at private residences where cases occurred, 6 gave typhoid fever histories as follows: Four years prior, 1 case; five years prior, 1 case; twelve years prior, 1 case; thirteen years prior, 1 case; twenty years prior, 1 case; sixty years prior, 1 case.

For 20 cases the newcomers were said to have never had typhoid fever and for 10 cases typhoid histories of the newcomers were not obtainable.

In the endeavor to learn what part was played by servants in the transmission of typhoid fever infection to their employers, the following data were obtained:

Seventy-one, or 13 per cent, of the 523 cases occurred at homes at which servants were employed. Of the last 306 cases investigated in 1906 and of which data regarding servants were obtained, 69, or 22 per cent, occurred at homes at which servants were employed. The difference in the percentage of cases occurring at homes where there were servants for the 1906 and the 1907 periods shows very well the difference in the class of people affected during the two periods.

Of the 71 cases occurring in the 1907 period at homes at which servants were employed, 3 were at homes where white servants were employed and resided, 1 was at a home where a white servant was employed, but who went to his own home at night, 23 were at homes where colored servants were employed and resided, and 44 at homes where colored servants were employed, but who went to their own homes at night.

Of the 97 servants who were employed at the homes of these 71 cases the typhoid histories were as follows:

White resident servants who had had typhoid fever (eighteen years prior)	1
White resident servants who had not had typhoid fever	3
White nonresident servants who had had typhoid	0
White nonresident servants who had not had typhoid	1
Colored resident servants who had had typhoid (six years prior)	1
Colored resident servants who had not had typhoid	33

Colored nonresident servants who had had typhoid fever (3, two years prior; 1, four years prior; 1, five years prior; 2, six years prior; 1, ten years prior)	8
Colored nonresident servants who had not had typhoid fever	41
Colored nonresident servants of whom typhoid histories were not obtained	9

Seventeen cases occurred at hotels, hospitals, or other public institutions at which there was a shifting of the servant population; so that accurate data regarding servants could not be obtained.

In no instance was a history obtained of the existence of typhoid fever cases at the homes of the nonresident servants during the three or four weeks prior to the onset of cases, nor was there any instance in which the information obtained indicated that the infection was conveyed by the servants to the members of employers' families.

There were 30 instances in which two or more cases developed in the same house, and in these 30 houses there were, all told, 73 cases. The largest number of cases occurring in one house was 4, and there were two such instances. Of these 73 cases, 34 were attributed to infection by direct contact, 7 to milk infection, and 32 to factors not determined.

Of the cases investigated by us in 1907, there were instances in which patients were cared for during the whole or a part of their illness in buildings at which there were grocery stores, bakeries, dairies, saloons, or restaurants, as follows:

Class of establishment:	Cases.
Grocery stores	10
Bakeries	4
Dairies	5
Saloons	4
Restaurants	5

In the majority of these instances some member of the family or other person in attendance on the patient was closely associated with the business; also in the majority of instances the precautions taken in the disposal of stools, urine, etc., were decidedly inefficient.

In considering the number of ways in which infection by personal contact may occur, it is easy to understand that many cases of typhoid fever in a large city may be due to infection by contact which can not be traced.

The time seems ripe and our knowledge of the subject sufficient to warrant the regarding of typhoid fever as being in large part a contact disease, and its prophylactic treatment as a quarantinable disease.

PROPHYLAXIS.

Of the total 670 cases investigated, 310, or 46.2 per cent, were treated at hospitals. One case was treated from the time of onset of illness at a place out of the District of Columbia. The time of admission to the hospital of cases considered as having contracted the infection in the District of Columbia was as follows:

	Cases.
Within five days after onset of illness	143
Within ten days after onset of illness	57
Within fifteen days after onset of illness	12
Within twenty days after onset of illness	3
Within twenty-five days after onset of illness	3
Within thirty days after onset of illness	1
Total	219

Of the 117 cases considered as undoubtedly having contracted the infection out of the District of Columbia, 78 were treated at hospitals; and of the 30 which were considered as almost certainly having contracted the infection out of the District of Columbia, 13 were treated at hospitals. Of these 91 imported cases treated at hospitals, 67 were taken from places out of the District of Columbia directly to hospitals for treatment and 24 were taken to hospitals after being treated at private residences in the District of Columbia as follows:

At private residences:	Cases.
One day	7
Two days	2
Three days	2
Five days	2
Six days	1
Seven days	2
Eight days	2
Nine days	2
Ten days	1
Twelve days	2
Twenty-four days	1
Total	24

This year, as in 1906, the good percentage of cases, particularly of the imported cases, treated at hospitals was encouraging, as the facilities for preventing the spread of infection are usually much better in hospitals than at private residences.

Of the 359 cases treated at private residences, 90 were attended by professional nurses. The treatment of stools and urine with disinfectants was considered efficient for 128 cases, inefficient for 180, of doubtful efficiency for 48, and the method not ascertained for 3.

Of the 180 patients whose stools and urine were inefficiently treated no attempt at disinfection was made for 65; the treatment was inefficient on account of the small quantity of disinfectant used for 58; and on account of a shortness of time of exposure of excreta to disinfectant for 57. For 48 of the patients the treatment of the stools and urine was considered of doubtful efficiency because the agent used for accomplishing the disinfection was of doubtful value.

The large number of instances in which the stools and urine of the patients were not properly disinfected shows the need of some radical action to be taken in regard to this matter.

Of other measures to prevent the spread of the infection, such as treatment of clothing, bedding, dishes, etc., used by the patients, the precautions carried out were considered efficient for 79, fairly efficient for 96, inefficient for 181, and not determined for 3.

Of the cases investigated by us in 1906, the treatment of the stools and urine of the patients cared for at private residences was as follows:

Treatment of stools and urine:	Cases.
Efficient	145
Inefficient	286
Of doubtful efficiency	51
Method not determined	10
Total	492

Thus, of the cases investigated by us in 1906 and in 1907 for the large majority cared for at private residences, the treatment with disinfectants of the stools and urine and the other measures to prevent the spread of infection from the patients were decidedly inefficient.

SUMMARY FOR 1907.

Six hundred and seventy cases investigated May 1 to November 1, 1907:

	Cases.	Percentage.
Infection contracted out of District of Columbia	174	25.97
Infection attributed to milk	48	7.17
Infection attributed to contact	102	15.22
Accounted for	324	48.36
Unaccounted for	346	51.64
Total	670	100.00

Or, considering only the 523 cases, of which 363 undoubtedly and 160 probably contracted the infection in the District of Columbia, we have—

	Cases.	Percentage.
Infection attributed to milk	48	9.18
Infection attributed to contact	102	19.50
Accounted for	150	28.68
Unaccounted for	373	71.32
Total	523	100.00

To contrast with the above figures we reprint the following summary of our findings in 1906 from Hygienic Laboratory Bulletin No. 35, page 57:

SUMMARY FOR 1906.

Eight hundred and sixty-six cases investigated June 1 to November 1, 1906:

	Cases.	Percentage.
Infection contracted out of District of Columbia	129	14.89
Infection attributed to milk or ice cream	85	9.81
Infection attributed to contact	54	6.23
Accounted for	268	30.93
Unaccounted for	598	69.07
Total	866	100.00

Or, considering only the 747 cases, of which 681 undoubtedly and 66 probably contracted the infection in the District of Columbia, we have—

	Cases.	Percentage.
Attributed to infection by milk or ice cream	85	11.30
Attributed to infection by contact	54	7.30
Accounted for	139	18.60
Unaccounted for	608	81.40
Total	747	100.00

The above figures are necessarily not exact, but are approximations based on the findings.

CONCLUSIONS.

1. Much of the typhoid fever in the District of Columbia is imported.

2. Many cases in the District of Columbia are contracted through contact with persons, or with articles handled or soiled by persons in the febrile stage of the disease. The especial prevalence of the disease among children in the District of Columbia probably has an important bearing on the spread of the infection by contact.

3. Infected milk is one of the important known factors in the spread of the disease in the District of Columbia.

4. The filtered Potomac River water during the typhoid season of 1907 (May to September) was according to present bacteriologic standards of good sanitary quality and so far as could be ascertained was not responsible for the spread of the infection.

In the typhoid season of 1907 there were about 200 cases less than in the 1906 period. This improvement in the situation suggests that the diminution in the amount of typhoid fever in the District of Columbia was due to the improvement in the quality of the drinking water as the result of sand filtration. Positive proof of this can not now be established. However, a careful study of typhoid fever in the District of Columbia for the next three to five years will throw much light on the rôle played by the Potomac River water in previous years.

RECOMMENDATIONS.

We recommend:

1. That all cases of typhoid fever and all cases of suspected typhoid fever shall be treated as contagious and dangerous to the community. This should include isolation, placarding, the prompt disinfection of the discharges, the patient's bedding, etc.

2. That laboratory facilities be provided free of cost to aid physicians in the early diagnosis of typhoid fever and also to determine when persons who have had the disease no longer menace the public health by discharging typhoid bacilli.

3. That all milk not "certified" or "inspected"[a] shall be pasteurized, under official surveillance, especially during the typhoid fever season.

4. The enactment of a law prohibiting the handling or sale of milk or milk products in any dwelling or in any structure so situated as to render contamination of these products especially liable.

5. The enactment of a law prohibiting the care of a case of typhoid fever in any house where foods or beverages liable to convey the infection are sold or prepared for sale.

6. That in order to furnish a water supply of a satisfactory grade of purity throughout the year, additional storage reservoirs shall be constructed, or a coagulant (alum) shall be used during periods of high turbidity.

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[a] For the definition of "certified" or "inspected" milk, see "The classification of market milk," by A. D. Melvin, Hygienic Laboratory Bulletin No. 41, p. 559.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

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*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No 22.—Chloride of zinc as a deodorant antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersuseptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Cr. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous zoological papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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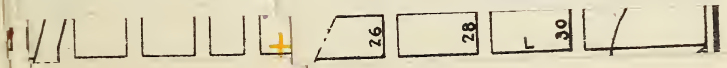

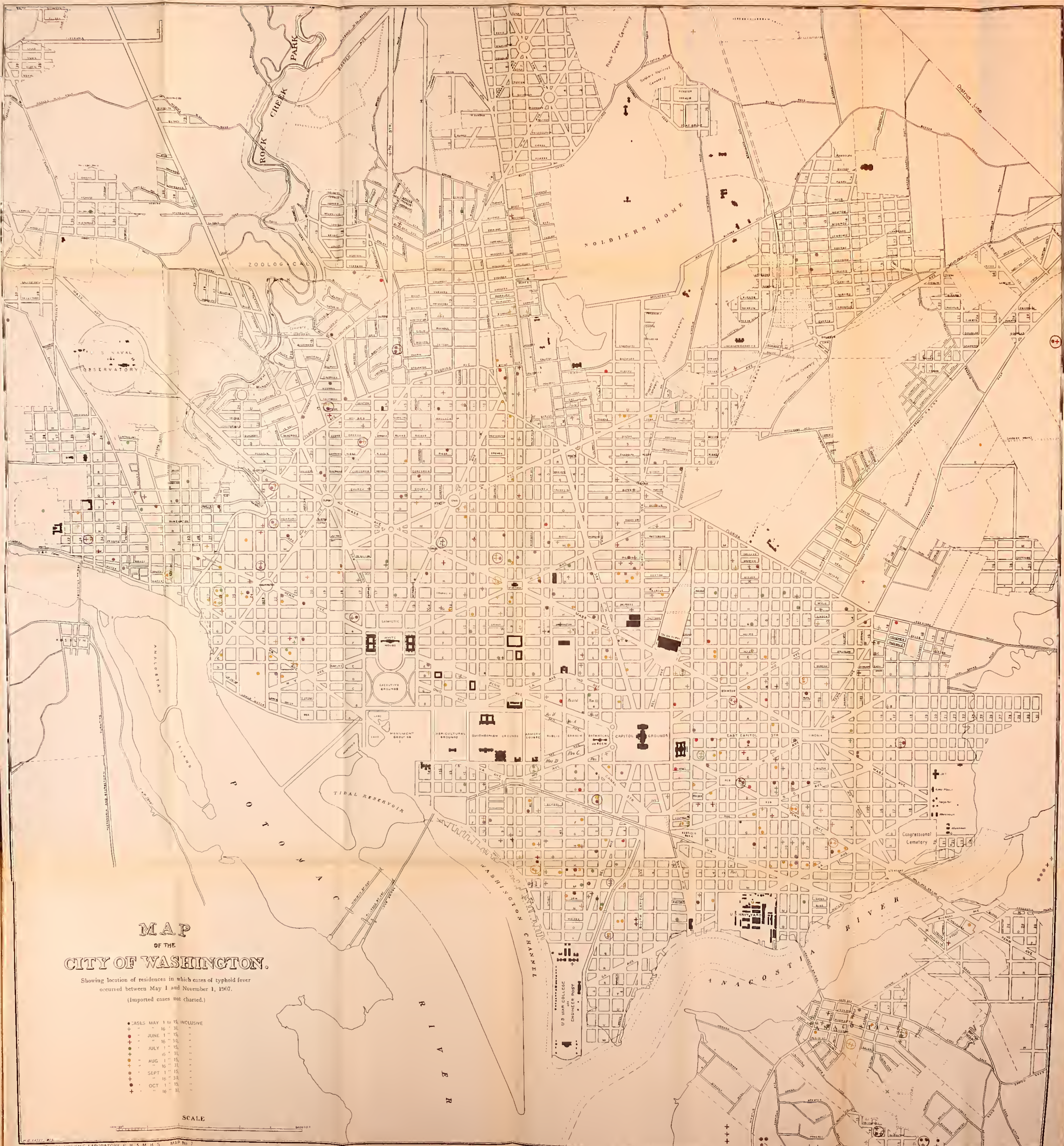

MAP OF THE CITY OF WASHINGTON.

Showing location of residences in which cases of typhoid fever occurred between May 1 and November 1, 1907.

(Imported cases not charted.)

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S. MAP No. 7

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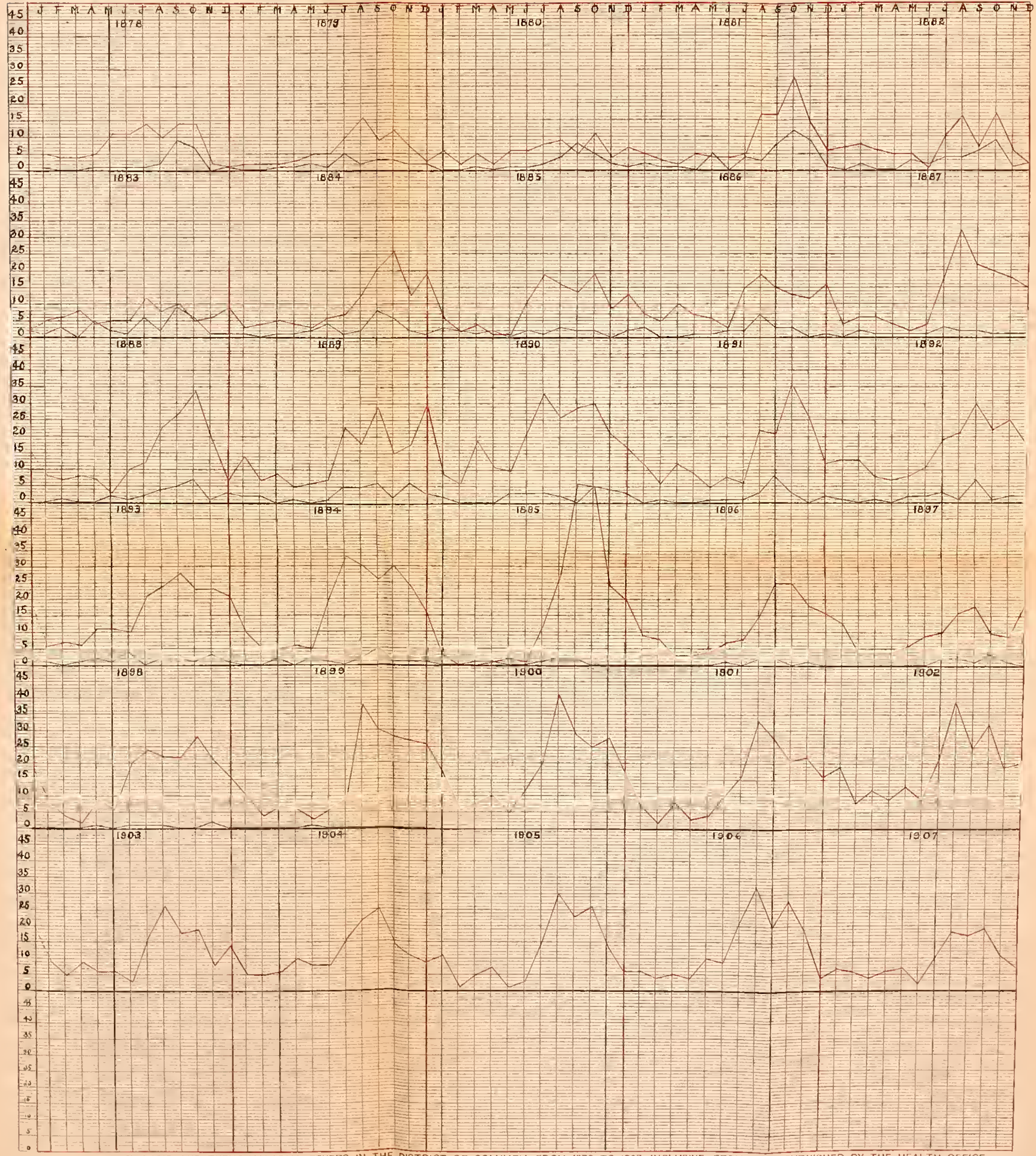
CHART No. 3



DEATHS FROM TYPHOID AND TYPHO-MALARIAL FEVERS IN THE DISTRICT OF COLUMBIA FROM 1878 TO 1907, INCLUSIVE, FROM DATA FURNISHED BY THE HEALTH OFFICE.

—— RED LINES INDICATE TYPHOID FEVER

—— BLACK LINES INDICATE TYPHO-MALARIAL FEVERS

THE NORRIS PETERS CO., WASHINGTON, D. C.

TREASURY DEPARTMENT

Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 45

JUNE, 1908

FURTHER STUDIES UPON ANAPHYLAXIS

By

M. J. ROSENAU

and

JOHN F. ANDERSON

WASHINGTON
GOVERNMENT PRINTING OFFICE
1908

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United States Public Health and Marine-Hospital Service.

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FURTHER STUDIES UPON ANAPHYLAXIS.[a]

By MILTON J. ROSENAU,
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and
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PART 1.—THE PERIOD OF INCUBATION.

In our first publication[b] from a limited series of experiments we found the "period of incubation," or the time necessary to elapse between the first and second injection, to be about ten days. We here give the results of three series of tests in order to clear up the following interesting details concerning the period of incubation: (1) Does the hypersusceptibility come on abruptly or gradually? (2) Do guinea pigs sensitized in the brain have a shorter period of incubation than those sensitized subcutaneously? (3) Is the period of incubation constant or variable?

TABLE No. 1.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1436	0.01 c. c. normal horse serum subcutaneously.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
1437	.....do	4	.....do	No symptoms.
1438	.....do	5	.....do	No symptoms.
1439	.....do	5	.....do	No symptoms.
1440	.....do	6	.....do	No symptoms.
1441	.....do	6	.....do	No symptoms.
1442	.....do	7	.....do	No symptoms.
1443	.....do	7	.....do	No symptoms.
1444	.....do	8	.....do	No symptoms.
1445	.....do	8	.....do	No symptoms.
1446	.....do	9	.....do	Slight symptoms.
1447	.....do	9	.....do	Slight symptoms.
1448	.....do	10	.....do	Marked symptoms.
1449	.....do	10	.....do	Marked symptoms.

---

[a] Manuscript submitted for publication May 5, 1908.

[b] Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the injection of horse serum. Hyg. Lab. Bul. No. 29, April, 1906, 95 p. Also Bul. No. 36, Further studies upon hypersusceptibility and immunity.

TABLE No. 2.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1363	0.01 c. c. normal horse serum into brain.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1364	.....do	2	.....do	No symptoms.
1365	.....do	3	.....do	No symptoms.
1366	.....do	4	.....do	Suggestive.
1367	.....do	5	.....do	Suggestive.
1368	.....do	7	.....do	Died in 25 minutes.
1370	.....do	8	.....do	Suggestive.
1369	.....do	9	.....do	Slight symptoms.
1371	.....do	10	.....do	Dead in 35 minutes.
1372	.....do	10	6 c. c. normal horse serum subcutaneously.	Mild symptoms.

TABLE No. 3.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1423	0.01 c. c. normal horse serum into brain.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
1424	.....do	4	.....do	No symptoms.
1425	.....do	5	.....do	No symptoms.
1426	.....do	5	.....do	No symptoms.
1428	.....do	6	.....do	No symptoms.
1429	.....do	6	.....do	No symptoms.
1427	.....do	7	.....do	No symptoms.
1430	.....do	7	.....do	Slight symptoms.
1431	.....do	8	.....do	Slight symptoms.
1432	.....do	8	.....do	Slight symptoms.
1433	.....do	9	.....do	Slight symptoms.
1434	.....do	9	.....do	Slight symptoms.
1435	.....do	10	.....do	Marked symptoms.

It will be seen from the above work that the period of incubation is about seven days or less in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. So far as may be judged, it therefore appears that the period of incubation is somewhat shorter in guinea pigs sensitized in the brain than those sensitized subcutaneously. It also seems quite evident that the sensitization comes on somewhat gradually. Judged by our own results and the work of others, the period of incubation is quite constant.

Lewis[a] states that the incubation period is not to be considered as abruptly terminating at a given day. He says that he has made an animal quite sick by the intracardial injection of 2 c. c. of serum on the sixth day after a toxine-antitoxin mixture.

---

[a] Lewis, Paul A.: The induced susceptibility of the guinea pig to the toxic action of the blood serum of the horse. Journ. Exper. Med., vol. 10, No. 1, Jan. 1, 1908, p. 1.

RELATION OF THE AMOUNT OF THE SENSITIZING DOSE TO THE TIME INTERVAL.

It seemed to us at one time in our work that animals injected with a large quantity of horse serum at the first injection had a more prolonged period of incubation or were less sensitive than guinea pigs given the usual minute amounts. We conjectured that this might have been due to the slow absorption of the serum, thus having somewhat the immunizing effect of repeated injections. We therefore prepared four series of guinea pigs: One sensitized with 0.01 c. c., a second with 0.1 c.c., a third with 1 c. c., and a fourth with 8 c. c. of normal horse serum. All of these animals were subsequently tested by intracerebral injections of the same normal horse serum.

So far as may be judged from this work, the period of incubation is not appreciably prolonged by a large sensitizing dose. Further, the animals sensitized with horse serum alone, remain so for a long period of time (245 days).

TABLE NO. 4.—*Relation of amount to sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1148	0.01 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Marked symptoms.
1149	.....do	17	.....do	Dead in 5 minutes.
1150	.....do	24	.....do	Severe symptoms.
1151	.....do	38	.....do	Severe symptoms.
1152	.....do	110	.....do	Dead in 6 minutes.
1155	.....do	155	.....do	Very severe symptoms.
1157	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.
1158	0.1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1159	.....do	17	.....do	Severe symptoms.
1160	.....do	24	.....do	Severe symptoms.
1161	.....do	38	.....do	Dead in 4 minutes.
1163	.....do	110	0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
1164	.....do	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1165	1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1166	.....do	17	.....do	Severe symptoms.
1167	.....do	24	.....do	Severe symptoms.
1168	.....do	24	.....do	Severe symptoms.
1169	.....do	110	.....do	Very severe symptoms.
1170	.....do	155	.....do	Very severe symptoms.
1162	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

TABLE NO. 4.—*Relation of amount to sensitization*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1172	8 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1173	.....do	17	.....do	Severe symptoms.
1174	.....do	24	.....do	Severe symptoms.
1175	.....do	38	.....do	Severe symptoms.
1177	.....do	110	0.5 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1176	.....do	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1178	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

PART 2.—THE SENSITIZING PRINCIPLE.

THE EFFECT OF HEAT UPON THE SENSITIZING ACTION OF HORSE SERUM.

In previous publications we reported that the toxic action of horse serum was not destroyed when the serum was heated to 60° C. for six hours, but is entirely destroyed at 100° C. in fifteen minutes. Further work on this subject leads us to conclude that the sensitizing effect of horse serum is gradually influenced by heat, and almost entirely disappears when the serum is heated to 100° C. for one hour. The pigs sensitized with small quantities of horse serum heated to 100° C. for one hour, when subsequently tested, developed very slight symptoms.

TABLE No. 5.—*Effect of heat upon sensitizing principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 60° C.			
1193	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 60° for 1 hour, subcutaneously.	25	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
	Heated to 70° C.			
1194	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 70° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
1195	.....do	25	0.25 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
	Heated to 80° C.			
1197	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 80° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Marked symptoms; died 2 days later.
1196	.....do	25	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
3242	.....do	39	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
3244	.....do	39	.....do	Severe symptoms.
3240	.....do	39	.....do	Severe symptoms.
	Heated to 90° C.			
1199	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 90° for 1 hour, subcutaneously.	25	.....do	No symptoms.
1198	.....do	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
3238	.....do	39	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 90 minutes.
3235	.....do	39	.....do	No symptoms.
3239	.....do	39	.....do	Marked symptoms.

TABLE NO. 5.—*Effect of heat upon sensitizing principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 100° C.			
1200	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1201	do	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1358	1 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	35	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
1359	do	35	do	Symptoms (?).
1360	do	35	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1361	do	35	6 c. c. normal horse (roan) serum subcutaneously.	Symptoms (?).
1362	do	35	do	Symptoms (?).

CAN GUINEA PIGS BE SENSITIZED IN THE BRAIN?

We were led to make the following experiments in view of a statement made by Besredka and Steinhardt[a] that guinea pigs were not sensitized when very small amounts are injected into the brain. Very minute amounts when injected into the brain in our experiments, as well as the few reported by Besredka and Steinhardt, proved negative.

However, guinea pigs may be readily sensitized by intracerebral injections, provided quantities of 0.0001 c. c. or more are used. We obtained negative results with sensitizing doses of 0.00001 c. c.

TABLE NO. 6.—*Intracerebral sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1202	0.25 c. c. normal horse (roan) serum into brain.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
1203	do	25	0.25 c. c. same serum into brain.	Marked symptoms.
1204	0.1 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Mild symptoms.
1205	do	25	0.25 c. c. same serum into brain.	Marked symptoms.
1207	0.01 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1206	do	25	0.25 c. c. same serum into brain.	Marked symptoms.
1208	0.001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1209	do	25	0.25 c. c. same serum into brain.	Slight symptoms.
1210	0.0001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Marked symptoms
1211	do	25	0.25 c. c. same serum into brain.	Slight symptoms.
1213	0.00001 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	No symptoms.
1212	do	25	0.25 c. c. same serum into brain.	No symptoms.

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[a] Ann. de l'Inst. Pasteur, vol. 21, 1907, p. 384.

GUINEA PIGS MAY BE SENSITIZED IN THE EYE.

So far as may be judged from the following experiment, it appears that normal horse serum dropped upon the eye of a guinea pig may sensitize it to a second injection of horse serum given after an interval of seventeen days.

TABLE No. 7.—*Ocular sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1340	1 drop normal horse serum into each eye.	17	6 c. c. normal horse (roan) serum intraperitcneally.	Mild symptoms.
1341	.....do	28	6 c. c. normal horse (roan) serum subcutaneously.	No symptoms.
1342	.....do	28	.....do	No symptoms.

ATTEMPTS TO SENSITIZE GUINEA PIGS WITH PURE PROTEINS.

We have assumed that it is a protein substance in the horse serum, egg-white, milk, vegetable extracts, etc., that we have tested which sensitizes guinea pigs and poisons them at the second injection. The substances used by us of course have a very complex composition, so that it became desirable to use solutions of pure proteins for these tests. We are indebted to Dr. Lafayette B. Mendel, New Haven, Conn., for a quantity of edestin and excelsin, which are protein substances obtained in a chemically pure crystalline state. Our tests with these two particular proteins resulted negatively, due probably to the difficulty of obtaining a satisfactory solution of them.

TABLE No. 8.—*Pure protein.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1393	0.1 gm. edestin subcutaneously	21	3 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1394	0.01 gm. edestin subcutaneously	21	.....do	No symptoms.
1395	0.001 gm. edestin subcutaneously.	21	.....do	No symptoms.
1396	0.1 gm. edestin subcutaneously	22	6 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1397	0.01 gm. edestin subcutaneously	22	.....do	No symptoms.
1398	0.001 gm. edestin subcutaneously.	22	.....do	No symptoms.
1399	0.1 gm. excelsin subcutaneously	21	3 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms (?).
1400	0.01 gm. excelsin subcutaneously	21	.....do	Symptoms (?).
1401	0.001 gm. excelsin subcutaneously	21	4 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms (?).
1402	0.1 gm. excelsin subcutaneously	22	6 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	No symptoms.
1403	0.01 gm. excelsin subcutaneously	22	.....do	No symptoms.

THE EFFECT OF BLOOD SERUM AND BRAIN OF SENSITIZED GUINEA PIGS UPON THE SENSITIZING SUBSTANCES.

Normal horse serum was mixed with an equal volume of the blood serum of a sensitive guinea pig and allowed to stand twenty-four hours at room temperature before the mixture was injected into a normal guinea pig.

Normal horse serum was also mixed with the brain substance of a sensitive guinea pig, well rubbed up in a mortar, and allowed to stand at room temperature twenty-four hours. The extract, strained through gauze, was injected subcutaneously into normal guinea pigs.

After a proper period of incubation these animals were tested and it was found that the anaphylactic phenomenon was in no wise modified by these procedures.

Table No. 9.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1406	1 c. c. mixture of serum of G. P. 9558 +normal horse (roan) serum, equal parts, subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	Severe symptoms.
1407	.....do................................	19	.....do.......................	Severe symptoms.
1405	1 c. c. mixture of brain of G. P. 9558+normal horse (roan) serum, equal parts, subcutaneously.	19	.....do.......................	Severe symptoms.
1408	.....do................................	19	.....do.......................	Severe symptoms.

Guinea pig No. 9558 received a subcutaneous injection of .25 c. c. of diphtheria toxine No. 7 + 1/340 c. c. antitoxic serum (Welc. 839) 61 days before being bled.

PART 3.—THE TOXIC PRINCIPLE.

THE EFFECT OF HEAT UPON THE TOXICITY OF HORSE SERUM.

It will be seen from the following tables that normal horse serum may be heated to 90° C. for one hour and still remain slightly toxic when injected into a sensitized guinea pig. Its toxicity, however, is evidently markedly affected. Heating to 70° C. for one hour does not seem to appreciably diminish its poisonous properties, but it appears to be affected at 80° for one hour. At 100° for one hour the toxicity apparently disappears.

Blood serum of course can not be heated to these high degrees without coagulation, and it is therefore necessary to dilute it in the proportion of one part of blood serum to three parts of distilled water. This dilution may then be heated to a high temperature without producing any visible change other than a slight opalescence.

It appears that there is a slight difference between the sensitizing and toxic principles in horse serum so far as resistance to heat is concerned. Serum heated to 100° C. for one hour retains some power of sensitization, but seems to lose its toxicity when given at the second injection. This difference may be more apparent than real, for exceedingly minute amounts are sufficient to sensitize guinea pigs, while a very large quantity of weakened serum would be necessary to produce symptoms. It must be remembered that in our experiments 20 c. c. of the dilution represents but 5 c. c. of the serum.

These facts must be considered in drawing conclusions from work upon split proteins, fractional precipitation, or other methods to isolate the sensitizing substance in pure form. A very minute amount of the original protein substance in horse serum clinging to the globulins or other substances modified by chemical methods might be sufficient to sensitize guinea pigs, whereas it would require very large amounts of such a modified protein to poison a sensitive animal.

TABLE NO. 10.—*The effect of heat upon the toxic principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 70° C. 1 hour.	
531	0.0006 c. c. tetanus toxine A+0.22 c. c. antitoxic horse serum (Ehrl.).	67	0.25 c. c. normal horse (roan) serum diluted 1:3, heated, concentrated, then injected into brain.	Marked symptoms.
533	0.0006 c. c. tetanus toxine A+0.24 c. c. antitoxic horse serum (Ehrl.).	67	.....do	Marked symptoms.
8496	0.24 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Cutter 1858).	69	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Dead in 40 minutes.
8495	.....do	69	.....do	Dead in 32 minutes.
			Heated to 80° C. 1 hour.	
8380	0.24 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (PD 08043).	54	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Very severe symptoms.
8494	0.24 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Cutter 1858).	42	.....do	Slight symptoms.
8857	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	67	.....do	Slight symptoms.
8886	0.245 c. c. toxine No. 42+1/150 c. c. antitoxic horse serum (Alex. A245).	67	.....do	Severe symptoms.
8890	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do	No symptons.
8883	0.245 c. c. toxine No. 42+1/440 c. c. antitoxic horse serum (Alex. A262).	67	.....do	No symptoms.
8872	0.245 c. c. toxine No. 42+1/660 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do	No symptoms.
			Heated to 90° C. 1 hour.	
8860	0.245 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (Str. 61S).	67	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
8880	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Mul. 2321).	67	.....do	Severe symptoms.
8878	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Mul. 2350).	67	.....do	Mild symptoms.
8880	0.245 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do	Slight symptoms.
8873	0.245 c. c. toxine No. 42+1/800 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do	Slight symptoms.

TABLE No. 10.—*The effect of heat upon the toxic principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 100° C. 1 hour.	
9260	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 38B).	42	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
9218	0.23 c. c. toxine No. 7+1/1100 c. c. antitoxic horse serum (Ldrl. 21C).	42	10 c. c. same dilution (=2.5 c. c. serum) subcutaneously.	No symptoms.
9215	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (Ldrl. 21).	42	0.25 c. c. same dilution (=2.5 c. c. serum) into brain.	No symptoms.
9210	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 19B).	42	.....do........................	No symptoms.
9343	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
			Next day 6 c. c. unheated normal horse (Frank) serum intraperitoneally.	Severe symptoms.
9344	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. dilution heated..........	No symptoms.
			Next day 6 c. c. unheated (Frank) serum.	Severe symptoms.

DOES HORSE SERUM DECREASE IN TOXICITY WITH AGE?

Besredka[a] states that freshly drawn horse serum is more toxic than the same serum thirty days old. Our own results are not in harmony with this conclusion, as may be seen from the following series.

TABLE No. 11.—*Effect of age upon toxicity of horse serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Serum 2 days old.	
8895	0.245 c. c. toxine No. 42+1/180 c. c. antitoxic horse serum (Cutter) subcutaneously.	67	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes.
8897	0.254 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Cutter).	67	.....do........................	Dead in 14 hours.
8871	0.245 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do........................	Dead in 2 hours.
8896	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter).	67	.....do........................	Very severe symptoms.
8858	0.245 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Hubbert).	67	.....do........................	Dead in 50 minutes.

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[a] Ann. de l'Inst. Pasteur, vol. 21, 1907, p. 777.

TABLE NO. 11.—*Effect of age upon toxicity of horse serum*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Same serum 15 days old.	
8856	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert)	112	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 40 minutes.
8859	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	112	.....do..........................	Dead in 40 minutes.
8860	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert).	112	.....do..........................	Very severe symptoms.
			Same serum 40 days old.	
9221	0.123 c. c. toxine No. 7+1/420 c. c. antitoxic horse serum (Alex. A283).	40	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 25 minutes.
9209	0.123 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Ldrl. 19).	40	.....do..........................	Dead in 27 minutes.
9257	0.123 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Ldrl. 30).	40	.....do..........................	Very severe symptoms.
			Same serum 68 days old.	
9370	0.23 c. c. toxine No. 7+1/1120 c. c. antitoxic horse serum (NY 306).	61	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 45 minutes.
9447	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (PD 08033).	61	.....do..........................	Dead in 85 minutes.
9375	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306).	61	.....do..........................	Severe symptoms.
			Same serum 91 days old.	
9460	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 11C).	72	6 c. c. normal horse (roan) serum subcutaneously.	Very severe symptoms.
9462	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 15B).	72	.....do..........................	Dead in 40 minutes.
9465	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 13).	72	.....do..........................	Dead in 90 minutes.
			Same serum 118 days old.	
9158	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Cutter 19734).	141	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 50 minutes.
9633	0.23 c. c. toxine No. 7+1/620 c. c. antitoxic horse serum (Alex. A249).	111	.....do..........................	Dead in 40 minutes.
9636	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249).	111	.....do..........................	Dead in 45 minutes.

THE EFFECT OF VARIOUS CHEMICAL SUBSTANCES UPON THE TOXICITY OF HORSE SERUM.

Further attempts were made to influence the toxic action of horse serum by treating the guinea pig with various chemicals.

In the following series the sensitized animals were given a subcutaneous injection of various substances the day before the second injection of serum. No favorable influence upon the anaphylactic state was obtained by the substances used, namely, pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chlorate, and calcium acetate.

TABLE No. 12.—*Effect of various chemicals.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
8545	0.24 c. c. toxine 42+1/640 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. pancreatin; next day 0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
8553	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. potassium oxalate; next day serum as above.	Dead in 5 minutes.
8552	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	0.5 gm. pepsin; next day serum as above.	Dead in 2 minutes.
8532	0.24 c. c. toxine 42+1/620 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. sodium sulphate; next day serum as above.	Dead in 3 minutes.
8534	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. magnesium sulphate; next day serum as above.	Dead in 2 minutes.
8389	0.24 c. c. toxine 42+1/370 c. c. antitoxic horse serum (PD 08033).	32	0.2 gm. peptone; next day serum as above.	Dead in 2 minutes.
8408	0.24 c. c. toxine 42+1/260 c. c. antitoxic horse serum (Cutter 1838).	32	0.1 gm. calcium chlorate; next day serum as above.	Dead in 2 minutes.
8535	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. calcium acetate; next day serum as above.	Dead in 3 minutes.

IODINE.

Obermayer and Pick[a] found that when the aromatic radicals of a protein are combined with various substances the protein loses the power to produce precipitins of closely limited specificity for the original species. Their results suggest that the aromatic groups of the molecule are most closely related to the species specificity.[b] This indicates that the striking specificity of proteins of different species depends upon the aromatic groups of the protein molecule, and Vaughan has found evidence that the toxicity of the proteins depends upon these same groups.

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[a] Obermayer, Fr., and Pick, E. P.: Ueber die chemischen Grundlagen der Arteigenschaften der Eiweisskörper. Wiener klin. Woch., vol. 19, 1906, p. 327.

[b] Wells, H. Gideon: The present status of our knowledge of the chemistry of the processes of immunity. Arch. Internal Med., vol. 1, No. 2, February 15, 1908, p. 262.

Fleischmann[a] also found that tryptic digestion destroys this characteristic species specificity.

We made several tests to determine the effect of iodine upon the toxic action of horse serum, and it so turned out in the preliminary experiments that the symptoms appeared to be profoundly modified, in the sense that they were either delayed or inhibited. We therefore tested a large number of guinea pigs to determine this point, but found that so far as the toxicity of horse serum was concerned at the second injection it is not appreciably modified by the iodine.

In the following series the iodine was added to horse serum in the proportion of 1.5 gm. per 25 c. c. of serum and 3 gm. potassium iodide to aid solution.

TABLE NO. 13.—*Iodine added to serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result
9826	0.23 c. c. toxine No. 7+1/250 c. c. antitoxic horse serum (P. I. Bur.).	42	6 c. c. normal horse (Frank) serum and iodine subcutaneously.	Dead in 50 minutes.
9837	0.23 c. c. toxine No. 7+1/210 c. c. antitoxic horse serum (Swiss USA).	42	.....do........................	Dead in 80 minutes.
9830	0.23 c. c. toxine No. 7+1/390 c. c. antitoxic horse serum (Mul. 2539).	42	.....do........................	Severe symptoms.
9832A	0.23 c. c. toxine No. 7+1/480 c. c. antitoxic horse serum (Mul. 2539).	42	.....do........................	Slight symptoms.
725	0.0004 c. c. tetanus toxine A+0.0075 c. c. antitetanic serum (Standard).	42	.....do........................	Mild symptoms.
9778	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Strn. 1351).	53	6 c. c. normal horse serum (Teddy) and iodine subcutaneously.	Dead in 65 minutes.
9713	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Scher. II).	66	.....do........................	Slight symptoms.
9743	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Strn. 1110).	53	.....do........................	Slight symptoms.
9182	0.23 c. c. toxine No. 7+1/140 c. c. antitoxic horse serum (Mul. 2352).	103	6 c. c. normal horse (roan) serum and iodine intraperitoneally.	Dead in 30 minutes.
9187	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Mul. 2362).	103	.....do........................	Dead in 45 minutes.
9189	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Mul. 2433).	103	.....do........................	Dead in 15 minutes.
9783	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan) serum and iodine subcutaneously.	Mild symptoms.

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[a] Zeit. f. klin. Med., vol. 59, 1906, p. 515.

TABLE NO. 13.—*Iodine added to serum*—Continued.

G.P. No.	First injection.	Interval in days.	Second injection.	Result.
9730	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan) serum and iodine subcutaneously.	Mild symptoms.
8551	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Alex. A249).	237	.....do........................	Mild symptoms.
9554	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Well. 7L841).	73	.....do........................	Dead in 80 minutes.

TABLE NO. 14.—*Iodine injected separately.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9782	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution) subcutaneously; next day 0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
9752	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Strn. 1110).	53	Iodine (3 c. c. Gram's solution) subcutaneously; 1 hour later 6 c. c. normal horse (Teddy) serum intraperitoneally.	Marked symptoms.
9772	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (6 c. c. Gram's solution) subcutaneously; 1 hour later normal horse serum (Teddy) intraperitoneally.	Marked symptoms.
9657	0.23 c. c. toxine No. 7+1/340 c. c. antitoxic horse serum (PD 08022).	64	Iodine (5 c. c. Gram's solution) subcutaneously; 1 hour later 0.2 c. c. normal horse (roan) serum into brain.	Dead in 3 minutes.
9781	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution); 1 hour later 0.1 c. c. same horse serum into brain.	Dead in 3 minutes.

TABLE NO. 15.—*Controls for tables Nos. 13 and 14.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9836	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Swiss).	42	6 c. c. normal horse serum (Frank) subcutaneously.	Very severe symptoms.
9832	0.23 c. c. toxine No. 7+1/570 c. c. antitoxic horse serum (Mul. 2539).	42	.....do........................	Very severe symptoms.
9773	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	52	6 c. c. normal horse serum (Teddy) subcutaneously.	Severe symptoms.
9770	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Strn. 1351).	52	.....do........................	Dead in 40 minutes.
9780	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	52	.....do........................	Dead in 60 minutes.

NITRITES.

A few further tests were made to determine the relation of methemaglobin producing substances, such as the nitrites, upon the symptoms. The following two guinea pigs were given a subcutaneous injection of sodium nitrite. In thirty minutes the exposed mucous membranes appeared distinctly blue, and the guinea pigs were then tested for susceptibility.

Controls showed that the quantity of nitrite used was not sufficient in itself to kill the guinea pigs.

TABLE No. 16.—*Sodium nitrite.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9173	0.23 c. c. toxine No. 7+1/360 c. c. antitoxic horse serum (Mul. 2439).	106	0.03 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum intraperitoneally.	Dead in 15 minutes.
9168	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (Mem. Inst.).	106	0.025 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes

ETHER.

Besredka[a] reported some interesting observations concerning the prevention of anaphylaxis by ether narcosis. He stated that if sensitive guinea pigs are etherized to the stage of complete relaxation, and while in this state injected intracerebrally with normal horse serum and the administration of ether continued a short while, (if the narcosis is well conducted) the animals continue to sleep after the injection, and at the end of about half an hour awake without presenting the least symptoms of anaphylaxis. If the guinea pig is tested on the following day it will be found to be immune.

Of eight guinea pigs upon which we tried this experiment with ether seven died, we believe, from the effects of the second injection of horse serum. It is our belief that pig No. 9581, which recovered, had masked symptoms while under the influence of the ether and probably would not have died anyhow, for we have a certain number of recoveries from the intracerebral injections of 0.2 c. c. of horse serum. It is true, however, that the narcosis masks the symptoms.

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[a] Annales de l'Institut Pasteur, Dec. 25, 1907, vol. 21, no. 12, p. 957.

The difference in our results may be accounted for by the difference in toxicity of the French and the American serums, or by differences in susceptibility of the animals used.

Table No. 17.—*Ether.*

G. P. No.	Treatment.	Result.
9419	0.23 c. c. toxine No. 7+1/440 c. c. antitoxic horse serum (Alex. A276). 55 days later tested as follows: The animal was first trephined, then deeply narcotized with ether, and in the stage of complete relaxation injected with 0.25 c. c. antitoxic horse serum (Mul. 2295) into the brain. The etherization was continued several minutes longer.	Died.
9376	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.1 c. c. same serum.	Died.
9374	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.25 c. c. same serum.	Died.
9175	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2439). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9174	0.23 c. c. toxine No. 7+1/450 c. c. antitoxic horse serum (Mul. 2429). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9678	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245.) 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Died in 10 minutes.
9581	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Recovered.
	Next day 6 c. c. normal horse (Frank) serum intraperitoneally..............	No symptoms.
9582	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 65 days later etherized and tested with 0.2 c. c. antitoxic horse serum (Lyons) into brain.	Died in few minutes.

ATTEMPTS TO NEUTRALIZE HORSE SERUM WITH THE BRAIN AND BLOOD OF SENSITIZED GUINEA PIGS.

We made further attempts to neutralize the toxic action of horse serum by first treating it with sensitized guinea pig serum and also with the brain substance of sensitized guinea pigs. These attempts all proved futile.

In the following experiments normal horse serum was mixed with equal parts of sensitized guinea pig serum, and kept at room temperature twenty-four hours.

The brain of a sensitive guinea pig was removed under aseptic precautions, mixed with about 20 c. c. of normal horse serum, ground up in a mortar, kept at room temperature for twenty-four hours, and then strained.

TABLE NO. 18.—*Action of guinea pig serum and brain.*

G. P. No.	First injection.	Interval in days.	Second injection	Result.
9337	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Dead in 4 minutes.
9340	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Very severe symptoms.
9336	0.23 c. c. toxine No. 7+1/1040 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Dead in 5 minutes.
9338	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Marked symptoms.

PART 4.—SPECIFICITY.

SPECIFIC NATURE OF "ANAPHYLACTIN."

In a previous publication[a] on the specific nature of anaphylaxis we demonstrated that guinea pigs may be in a condition of anaphylaxis to three protein substances at the same time. Hypersusceptibility to each protein is manifested by a second injection of the corresponding protein. The three reactions may be obtained in the same guinea pig within a short space of time and are as distinct and specific as three separate infectious diseases.

We now bring forward experimental data proving that the substance in the blood serum of sensitized guinea pigs, known as anaphylactin, is also specific in the same sense. In the following table it will be seen that by transferring the blood serum of guinea pigs sensitized to horse serum, egg white, and milk three separate and distinct reactions were obtained in the guinea pig into which this serum was transferred:

TABLE No. 19.—*The specific nature of anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9619	0.23 c. c. toxine No 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously. 56 days later 1 c. c. cow's milk subcutaneously. 1 day later 1 c. c. egg white subcutaneously. 22 days later bled; blood defibrinated centrifuged, and serum used to inject G. P. No. 9619A.			
9619A	7 c. c. serum of G. P. 9619 subcutaneously.	2	6 c. c. milk intraperitoneally.... 2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Mild symptoms. Dead in 5 minutes.
9620	Same treatment as G. P. No. 9619.			
9620A	8 c. c. serum of G. P. 9620 subcutaneously.	2	6 c. c. milk intraperitoneally.... 2 days later 3 c. c. egg white intraperitoneally. 2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Slight symptoms. Mild symptoms. Dead in 4 minutes.
9621	0.23 c. c. toxine No. 7+1/580 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9621A	6.5 c. c. serum of G. P. 9621 subcutaneously.	2	6 c. c. milk intraperitoneally.... 2 days later 6 c. c. saturated solution egg white intraperitoneally. 2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Slight symptoms. Marked symptoms. Very severe symptoms.
9624	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9624A	7 c. c. serum of G. P. 9624 subcutaneously.	2	6 c. c. milk intraperitoneally.... 2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Slight symptoms. Dead in 5 minutes.

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[a] Rosenau, M. J., and Anderson, John F.: The specific nature of anaphylaxis. Jour. Infec. Dis., vol. 4, No. 4, Nov., 1907, pp. 552–557.

EFFECT OF IODINE UPON SPECIFICITY.

In the following series we not only tested guinea pigs treated with horse serum to find out whether they react after an interval to chicken serum, and vice versa, but also to ascertain whether the presence of iodine in the sensitizing dose modifies the specific nature of the reaction. The results plainly show that under the conditions of the experiment the iodine had no modifying action upon the power of these serums to sensitize against a second injection of homologous serum and also had no power to impair the specific nature of the phenomenon.

Table No. 20.—*Effect of iodine on specificity.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1613	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. chicken serum into brain.	Severe symptoms.
1604	.....do............................	19	.....do..........................	Severe symptoms.
1615	2 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	No symptoms.
1616	.....do............................	19	.....do..........................	No symptoms.
1617	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	.....do..........................	No symptoms.
1618	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do..........................	Severe symptoms.
1619	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do..........................	Dead in 4 minutes
1620	.....do............................	19	0.2 c. c. chicken serum into brain.	No symptoms.
1621	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do..........................	No symptoms.
1622	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do..........................	No symptoms.

RELATION BETWEEN MILKS OF VARIOUS SPECIES.

HUMAN VERSUS COW MILK.

Eight guinea pigs were sensitized by the subcutaneous injection of 1 c. c. of human milk. After an appropriate interval they were tested with cow's milk without response.

A short time later they were again tested with human milk. This time most of them showed severe symptoms.

This series again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter in human and in cow's milk.

TABLE NO. 21.—*Human versus cow milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1375	1 c. c. human milk subcutaneously.	37	6 c. c. cow's milk intraperitoneally.	No symptoms.
			2 days later 10 c. c. human milk subcutaneously.	No symptoms(?).
1376	.....do.....................	37	6 c. c. cow's milk subcutaneously...	No symptoms.
			4 hours later 10 c. c. human milk intraperitoneally.	Very severe symptoms.
1377	.....do.....................	37	6 c. c. cow's milk subcutaneously...	No symptoms.
			4 hours later 10 c. c. human milk intraperitoneally.	Very severe symptoms.
1378	.....do.....................	37	6 c. c. cow's milk intraperitoneally..	No symptoms.
			2 days later 10 c. c. human milk intraperitoneally.	No symptoms(?).
1401	.....do.....................	24	6 c. c. cow's milk intraperitoneally..	No symptoms.
			1 day later 5 c. c. human milk intraperitoneally.	Very severe symptoms.
1402	.....do.....................	24	6 c. c. cow's milk intraperitoneally..	No symptoms.
			1 day later 5 c. c. human milk intraperitoneally.	Very severe symptoms.
1403	.....do.....................	24	6 c. c. cow's milk intraperitoneally..	No symptoms.
			1 day later 5 c. c. human milk intraperitoneally.	Very severe symptoms.
1404	.....do.....................	24	6 c. c. cow's milk intrapertioneally..	No symptoms.
			1 day later 5 c. c. human milk intraperitoneally.	Very severe symptoms.

TABLE NO. 22.—*Sheep milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1450	1 c. c. sheep's milk subcutaneously.	26	6 c. c. cow's milk subcutaneously.	No symptoms.
1451	.....do.....................	26	.....do.....................	No symptoms.
1452	.....do.....................	26	6 c. c. cow's milk intraperitoneally.	Marked symptoms.
1453	.....do.....................	26	.....do.....................	Severe symptoms.
1454	.....do.....................	26	.....do.....................	Slight symptoms.
1455	.....do.....................	26	.....do.....................	Dead in 25 minutes.

TABLE NO. 23.—*Dog milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1600	0.5 c. c. dog milk subcutaneously...	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1601	.....do.....................	17	.....do.....................	No symptoms.
1602	.....do.....................	17	.....do.....................	No symptoms.
1603	1 c. c. dog milk subcutaneously.....	17	.....do.....................	No symptoms.
1604	.....do.....................	17	.....do.....................	No symptoms.
1605	.....do.....................	19	.....do.....................	No symptoms.
1606	.....do.....................	19	.....do.....................	No symptoms.
1607	.....do.....................	19	.....do.....................	No symptoms.

In the following series the dog milk was first treated with iodine and potassium iodide before it was injected subcutaneously.

TABLE NO. 24.—*Dog milk+iodine versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1608	0.5 c. c. dog milk+iodine subcutaneously.	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1609	.....do	17	.....do	No symptoms.
1610	.....do	17	.....do	No symptoms.
1611	1 c. c. dog milk+iodine subcutaneously.	17	.....do	No symptoms.
1612	.....do	17	.....do	No symptoms.

RELATION BETWEEN EGG ALBUMENS OF VARIOUS SPECIES.

TABLE NO. 25.—*Hen egg white versus duck egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1408	1 c. c. saturated solution hen egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution duck egg white in salt solution (0.85) intraperitoneally.	Mild symptoms.
1409	.....do	21	.....do	Mild symptoms.
1410	.....do	21	.....do	Mild symptoms.
1411	.....do	21	.....do	No symptoms.

TABLE NO. 26.—*Duck egg white versus hen egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1413	1 c. c. saturated solution duck egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 30 minutes.
1414	.....do	21	.....do	Dead in 35 minutes.
1415	.....do	21	.....do	Very severe symptoms.
1416	.....do	21	.....do	Very severe symptoms.
1417	.....do	21	.....do	Very severe symptoms.

TABLE NO. 27.—*Guinea hen egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2040	1 c. c. saturated solution guinea hen egg white in salt solution (0.85) subcutaneously.	20	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 13 minutes.
2041	.....do	20	.....do	Very severe symptoms.
2042	.....do	20	.....do	Very severe symptoms.
2043	.....do	20	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2044	.....do	20	.....do	Marked symptoms.

NOTE.—The crane egg used in these experiments was from a Demoiselle crane and kindly furnished us by Dr. Frank Baker.

TABLE NO. 28.—*Pigeon egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2035	1 c. c. saturated solution pigeon egg white in salt solution (0.85) subcutaneously.	21	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	No symptoms.
2036	.....do	21	.....do	No symptoms.
2037	.....do	21	.....do	No symptoms.
2038	.....do	21	6 c. c. saturated solution of crane egg white in solt solution (0.85) intraperitoneally.	Very severe symptoms.
2039	.....do	21	.....do	Marked symptoms.

TABLE NO. 29.—*Goose egg white versus hen and turkey egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2030	1 c.c. saturated solution goose egg white in salt solution (0.85) subcutaneously.	38	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2031	.....do	38	.....do	Very severe symptoms.
2032	.....do	38	.....do	Marked symptoms.
2033	.....do	38	6 c. c. saturated salt solution turkey egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2034	.....do	38	.....do	Severe symptoms.

Table No. 30.—*Turkey egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2025	1 c. c. saturated solution turkey egg white in salt solution (0.85) subcutaneously.	35	6 c. c. saturated solution of hen egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2026	.....do	35	.....do	Dead in 70 minutes.
2027	.....do	35	.....do	Dead in 70 minutes
2028	.....do	35	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Severe symptoms.
2029	.....do	35	.....do	Severe symptoms.
2030	.....do	35	6 c. c. saturated solution of turkey egg white in salt solution (0.85) intraperitoneally.	Dead in 20 minutes.

PART 5.—ANAPHYLACTIN.

THE PRESENCE OF ANAPHYLACTIN DURING THE PERIOD OF INCUBATION.

It is of some interest to determine just when the substance called *anaphylactin* by Gay and Southard appears in the blood of a sensitized guinea pig, particularly whether its presence may be demonstrated during the period of incubation.

A series of guinea pigs was therefore sensitized by the subcutaneous injection of 1/100 c. c. of normal horse serum. On each succeeding day two guinea pigs of this series were bled and the serum obtained by whipping and centrifugation. This serum was then injected into normal pigs. In twenty-four hours these pigs were tested by the injection of 6 c. c. of horse serum intraperitoneally. In the latter part of the series forty-eight hours were allowed to elapse before the pigs were tested.

It will be seen from Tables 31 and 32, which give our work on this factor in detail, that no indication of anaphylactin appeared in the blood of sensitized guinea pigs until the tenth day.

Table No. 31.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1343A	2 c. c. subcutaneously serum G. P. 1343, which was bled *1 day* after subcutaneous injection of 0.01 c. c. normal horse serum.	1	0.25 c. c. normal horse serum into brain.	No symptoms.
1343B	......do	1	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1344A	4 c. c. serum G. P. 1344, which was bled *2 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1345A	3 c. c. serum G. P. 1345, which was bled *3 days* after injection of 0.01 c. c. normal horse serum.	1	......do	No symptoms.
1345B	......do	1	......do	No symptoms.
1346A	2.5 c. c. serum G. P. 1346, which was bled *4 days* after injection of 0.01 c. c. normal horse serum.	1	......do	No symptoms.
1346B	......do	1	......do	No symptoms.

TABLE No. 31.—*Anaphylactin during the period of incubation*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1347A	3 c. c. serum G. P. 1347, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1347B	.....do	1	.....do	No symptoms.
1348A	5 c. c. serum G. P. 1348, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1348B	.....do	1	.....do	No symptoms.
1349A	4 c. c. serum G. P. 1349, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1350A	5 c. c. serum G. P. 1350, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1351A	6 c. c. serum G. P. 1351, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1353A	8 c. c. serum G. P. 1353, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do	Marked symptoms.
1354A	6.5 c. c. serum G. P. 1354, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Slight symptoms.
1355A	6.5 c. c. serum G. P. 1355, which was bled *12 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Slight symptoms.
1355B	1 c. c. same serum	2	.....do	Marked symptoms.
1356A	3 c. c. serum G. P. 1356, which was bled *13 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1356B	2.5 same serum	2	.....do	Mild symptoms.
1357A	3 c. c. serum G. P. 1357, which was bled *14 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Mild symptoms.
1357B	2.5 same serum	3	.....do	Severe symptoms.

The first indication of anaphylactin appeared in the above series on the tenth day, that is, just about the time necessary to render guinea pigs sensitive. It is also evident that forty-eight hours is a better interval than twenty-four for the purpose of demonstrating the presence of anaphylactin in guinea-pig serum.

Our interest in this subject led us to sensitize another series of guinea pigs with 1/100 c. c. normal horse serum subcutaneously, but to test for the presence of anaphylactin by injections into the brain.

TABLE NO. 32.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1380A	6 c. c. subcutaneously serum G. P. 1380, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
1381A	4 c. c. intraperitoneally serum G. P. 1381, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1382A	4 c. c. intraperitoneally serum G. P. 1382, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1383A	5 c. c. intraperitoneally serum G. P. 1383, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1384A	5 c. c. intraperitoneally serum G. P. 1384, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1385A	5 c. c. intraperitoneally serum G. P. 1385, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1386A	5 c. c. intraperitoneally serum G. P. 1386, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	No symptoms.
1387A	5 c. c. intraperitoneally serum G. P. 1387, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	Slight symptoms.
1388A	5 c. c. intraperitoneally serum G. P. 1388, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	Slight symptoms.
1389A	6 c. c. intraperitoneally serum G. P. 1389, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	Marked symptoms.
1390A	3.5 c. c. intraperitoneally serum G. P. 1390, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	Mild symptoms.
1391A	5 c. c. intraperitoneally serum G. P. 1391, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	2	.....do......................	Mild symptoms.
1391B	Hemaglobin intraperitoneally of G. P. 1391.	2	.....do......................	No symptoms.
1391C	Red corpuscles of G. P. 1391 intraperitoneally.	2	.....do......................	No symptoms.

In the above series the anaphylactin appeared in the blood of sensitized guinea pigs on the ninth day.

ANAPHYLACTIN IN MAN AND OTHER ANIMALS.

The presence of anaphylactin having been demonstrated in the blood serum of sensitized guinea pigs, it is interesting to know whether man and other animals that have received a previous injection of horse serum also contain a similar substance.

So far as may be judged from the following limited experiments upon the subject, we have been unable to demonstrate a similar property in the blood serum of man, the monkey, rabbit, and the cat when tested upon guinea pigs.

TABLE NO. 33.—*Anaphylactin in man, monkey, rabbit, and cat.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Man.			
1310	1 c. c. normal human serum (CWC) subcutaneously.	44	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
280	5 c. c. human serum (LLL). 7 years prior this man had been injected with 12 c. c. antitoxic horse serum.	1¾	.....do........................	No symptoms.
1311	0.4 c. c. human serum (JWT). 1 year prior this man had been injected with 5,000 units antitoxic horse serum.	34	.....do........................	Slight symptoms.
1312	1.5 human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	36	.....do........................	Marked symptoms.
1313	0.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do........................	Mild symptoms.
1314	1.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do........................	Slight symptoms.
1379	10 c. c. human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	2½	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Monkey.			
290	3.5 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅚	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
290A	3 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅚	.....do........................	No symptoms.

TABLE No. 33.—*Anaphylactin in man, monkey, rabbit, and cat*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Cat.			
285	3 c. c. cat serum. 41 days prior this cat had been injected with 2 c. c. normal horse serum.	$1\frac{5}{6}$	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
	Rabbit.			
1377	8 c. c. rabbit serum. This rabbit had been frequently injected with normal horse serum.	$2\frac{1}{2}$	....do....................	No symptoms.
1378	.....do...........................	$2\frac{1}{2}$	0.2 c. c. normal horse (roan) serum into brain.	No symptoms

It will be noticed that a few of these guinea pigs showed symptoms, but in every case an interval of at least two weeks elapsed between the first and the second injections. The slight reactions obtained were probably those which occur when serums of different species are used at the first and the second injections (heterologous serums, see Hyg. Lab. Bull. 29, p. 55, and Bull. 36, p. 25).

ANAPHYLACTIN IN IMMUNE GUINEA PIGS.

We submit some further work bearing upon the question whether guinea pigs "immunized" against the phenomenon of anaphylaxis are in a refractory state or have returned to the normal or are really immune.

We first made a few experiments to determine whether guinea pigs immunized by repeated injections of small quantities of horse serum contain anaphylactin in their blood. In this series the guinea pigs received ten injections of 2 c. c. normal horse serum subcutaneously covering a period of seventeen days. Eighteen days following the last injection the pigs were first tested for immunity and then bled.

It developed from the following six guinea pigs that ten repeated subcutaneous injections (of 2 c. c. each) during the course of seventeen days were not sufficient to completely immunize the guinea pigs, for those tested developed slight symptoms. Indications of anaphylactin were demonstrated in the blood serum of five of the six pigs of this series. However, it must be noted that when bled these animals were certainly immune from the last treatment, as we and others have shown that when a sensitive guinea pig responds to a second injection immunity is quickly established. (See G. P. No. 671, Table 36.)

TABLE NO. 34.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1370A	6 c. c. serum G. P. 1370, which had received 10 subcutaneous injections 2 c. c. normal horse serum in a period of 17 days.	2	6 c. c. normal horse serum intraperitoneally.	Mild symptoms.
1373A	6 c. c. serum G. P. 1373, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Mild symptoms.
1369A	6 c. c. serum G. P. 1369, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1371A	6 c. c. serum G. P. 1371, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Slight symptoms.
1374A	6 c. c. serum G. P. 1374, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1372A	6 c. c. serum G. P. 1372, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
671	0.0006 c. c. toxin A+1/1538 c. c. antitoxic horse serum (PD 09755).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Very severe symptoms.
			Next day 0.2 c. c. normal horse (roan) serum into brain. 30 minutes later bled for serum.	No symptoms.
671A	5 c. c. serum of G. P. 671T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.
666	0.0006 c. c. toxin A + .0002 gm. antitetanic serum (Tizzoni).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally. Next day bled for serum.	Very severe symptoms.
666A	6 c. c. serum of G. P. 666T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms

The following series of guinea pigs is a more conclusive test that anaphylactin may be demonstrated in the blood serum of immune guinea pigs.

Guinea pigs were first immunized by repeated subcutaneous injections of 5 c. c. normal horse serum. The pigs received from 6 to 12 such injections, amounting to 30 to 60 c. c. About eight months after this treatment the pigs were tested by intracerebral injections, to which some responded with slight symptoms. Two of them showed no symptoms at all. While all the pigs were not completely immunized by the first treatment of subcutaneous injections, they were certainly rendered immune by the second treatment, given eight months later. Two hours after the second treatment the guinea pigs were bled, the blood centrifugated, the clear serum pipetted off and injected subcutaneously into normal guinea pigs. These normal pigs so treated were tested two days later by intracerebral injections, and all of them showed symptoms.

TABLE No. 35.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1180	6 injections, each 5 c. c. normal horse (roan) serum subcutaneously, covering period of 18 days.	249 after last injection	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms
1180A	9 c. c. serum G. P. 1180 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1181	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1181A	8 c. c. serum G. P. 1181 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1182	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1182A	8 c. c. serum G. P. 1182 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1183	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1183A	12 c. c. serum G. P. 1183 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1186	7 injections, each 5 c. c. normal horse (roan) serum, in 21 days.	246	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1186A	8 c. c. serum G. P. 1186 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1187	8 injections, each 5 c c. normal horse (roan) serum, in 25 days	242	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1187A	9 c. c. serum G. P. 1187 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1188	9 injections, each 5 c. c. normal horse (roan) serum, in 28 days.	239	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1188A	8 c. c. serum G. P. 1188 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1189	10 injections, each 5 c. c. normal horse (roan) serum, in 32 days.	235	0.2 c. c. normal horse (roan) serum into brain. 2 hours latter bled for serum.	Slight symptoms.
1189A	5 c. c. serum G. P. 1189 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1190	11 injections, each 5 c. c. normal horse (roan) serum, in 35 days.	232	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Mild symptoms.
1190A	9 c. c. serum G. P. 1190 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1191	12 injections, each 5 c. c. normal horse (roan) serum, in 39 days.	228	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1191A	9 c. c. serum G. P. 1191 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.

It is therefore plain that anaphylactin does exist in the blood serum of immune guinea pigs.

We add the following examples showing that the presence of anaphylactin may not always be demonstrated. In these experiments the blood serum was collected in one of two ways. From guinea pigs Nos. 1000 to 1007, inclusive, the blood was drawn from the heart, allowed to clot at room temperature, and the serum pipetted off next morning. In guinea pigs Nos. 1138 to 1147, inclusive, the blood was drawn from the large vessels of the neck, defibrinated by whipping, centrifugated, and the clear supernatant serum used to inject into the normal guinea pig.

Perhaps the long interval may account for the negative results.

TABLE NO. 36.—*Anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1000	4 c. c. normal guinea pig serum subcutaneously.	26	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1001	1.5 c. c. serum G. P. 8038, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No.9+1/540 c. c. antitoxic horse serum (NY 13C).	26	.....do	No symptoms.
1002	2 c. c. serum G. P. 8048, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9+1/360 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1003	3.5 c. c. same serum	26	.....do	No symptoms.
1004	4 c. c. serum G. P. 8051, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/400 antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1005	4 c. c. serum G. P. 8045, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/560 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1006	4 c. c. same serum	26	.....do	No symptoms.
1007	6 c. c. serum G. P. 8050, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/320 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1138	1.5 c. c. serum G. P. 8128, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	16	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1139	3.5 c. c. same serum	16	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1140	1.5 c. c. serum G. P. 8127, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.

TABLE NO. 36.—*Anaphylactin*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1141	4 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1142	1.5 c. c. serum G. P. 8003, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/420 c. c. antitoxic horse serum (NY 1013) and bled 99 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1143	20 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1144	1.5 c. c. serum G. P. 8126, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1145	3 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1146	4 c. c. serum G. P. 7846, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/310 c. c. antitoxic horse serum (Alex. spl. 192) and bled 139 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Very severe symptoms.
1147	1.5 c. c. same serum	19	do	Severe symptoms.

TABLE NO. 37.—*Anaphylactin—Miscellaneous.*

G.P. No.	First injection.	Interval in days.	Second injection.	Result.
696T	0.0006 c. c. tetanus toxine A + 0.0025 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
695T	0.0006 c. c. tetanus toxine A + 0.002 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
711T	0.0006 c. c. tetanus toxine A + 0.0075 c. c. antitoxic horse serum (Standard T2).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Half an hour later above 3 pigs bled: blood mixed, defibrinated, and 12 c. c. of serum obtained.			
1392	12 c. c. blood serum of above 3 guinea pigs intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.

PART 6.—MECHANISM.

LESIONS.

Gay and Southard,[a] 1907, found in guinea pigs dying from a second injection of serum, and in those which had severe symptoms and were later chloroformed, lesions which are interpreted as explaining the mechanism of anaphylaxis. They state that "the study of the histopathology of this serum disease shows us that we have to deal with an intricate cell reaction demonstrable by definite cell lesions." Considerable hemorrhages, rather definitely localized, are the characteristic gross lesions. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically there are, in addition to the naked-eye hemorrhages, minute interstitial and oozing hemorrhages. They also found fttay changes in voluntary muscle fiber, heart muscle fiber, and in nerve fiber.

That the conjestion and dilatation of the blood vessels found in the abdominal cavity and the hemorrhages upon the mucosa of the stomach are not characteristic of death due to anaphylaxis is evident from the fact that we have found that in violent death produced by large subcutaneous injections of chloral cyanhydrin or hydrocyanic acid there are somewhat similar congestions and hemorrhages.[b] Further, we have lately had the opportunity to examine a guinea pig whose death was caused by suffocation in an atmosphere of carbon dioxid. In the stomach and lungs of this guinea pig lesions were found that, so far as the congestion and hemorrhages are concerned, were somewhat similar to those described in guinea pigs dying from a second injection of horse serum.

We were especially struck by the fact that the macroscopic congestions and hemorrhages may be absent in guinea pigs poisoned by intracerebral injections.

Further, this congestion and dilatation of the vessels of the abdominal cavity is well known to occur in shock and other states.

We were also unable to confirm Gay and Southard's findings in regard to the fatty changes.

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[a] Journ. Med. Research, May, 1907, p. 143.

[b] Rosenau, M. J., and Anderson, John F.: A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing on experimental gastric ulcer. Journ. Infec. Dis., vol. 4, No. 1, Jan., 1907, p. 1–7.

We studied a large number of pigs in which death occurred within thirty minutes of the second injection of serum; also, a moderate number which were killed by chloroform or otherwise from one to four hours after the second injection.

We are indebted to our colleague Dr. W. W. Miller, United States Public Health and Marine-Hospital Service, for the following studies upon the post-mortem appearances and pathology of the tissues of guinea pigs dead of anaphylaxis.

The most noticeable lesion to the naked eye is the marked dilatation of the small veins and capillaries of the body, but most noticeable in those of the abdominal viscera. Associated with this in about 25 per cent of the cases are hemorrhages in the mucosa of the stomach and, more rarely, of the intestines. Minute hemorrhages 1 mm. in size are occasionally observed on the surface of the lungs; no hemorrhages of the heart muscle, spleen, pericardium, or striped muscle, as described by Gay and Southard, have been seen.

For microscopic study the tissues from 16 guinea pigs were utilized. These pigs had received the second dose of serum in three ways, viz, by subcutaneous, intraperitoneal, and intracranial injection. Material was selected from all the viscera and from the striped muscles and nervous systems. Particular attention was given to the study of tissues for the fatty changes described by Gay and Southard. For this purpose sections made with the freezing microtome were used to a great extent, as it is generally recognized that fresh material is superior to that prepared and sectioned in the customary way in celloidin and paraffin, although it must be admitted, as Gay and Southard contend, that such preparations are not as permanent or suitable for micro-photographic purposes. For a general study of microscopic changes tissues were fixed in 10 per cent formalin, in formalin and alcohol (5 and 85 per cent), and Zenker.

For fatty degeneration, fresh tissue and tissue fixed for twenty-four hours in 5 to 10 per cent watery solution of formalin was used and sectioned with the freezing microtome; for nerve tissue, formalin 5 to 10 per cent, Orth's fluid, and Müller's fluid.

As a stain for general purposes, hematoxylin and eosin were used.

For staining fat, Marchi's method was carried out as follows: Fixation in 10 per cent formalin and Müller's fluid six to ten days, in Marchi's mixture six to ten days; kept throughout in the dark; then washed twenty-three hours in running water; hardened in alcohol and ether celloidin as quickly as possible. Clove oil celloidin was not used, as it causes general blackening.

For frozen sections of tissue fixed in 10 per cent formalin for twenty-four hours, the admirable method so highly recommended by Schmörl was used, viz, sections placed in Marchi fluid in closed vessels in the paraffin oven for one-half to one hour, washed quickly in water, and mounted in glycerin or dehydrated over night in alcohol and mounted in balsam.

As controls in this work, tissue was used from normal pigs, from a pig dead of puerperal sepsis with marked fatty changes in liver and kidney, and from pigs killed with diphtheria toxine. Sati's method of osmization was also used with some of the specimens.

Fresh and formalinized tissue was stained by the admirable method of Torrain Smith (nile blue sulphate); also, Sudan III.

Results.—In sections stained for general study the sole difference from normal tissue consists in the marked dilatation of the veins and capillaries, especially of the stomach and intestines, accompanied by extravasation of blood at points where the vessels are ruptured. The thin-walled veins of the mucosa of the stomach are often greatly distended. In the few instances where a ruptured point in the vessel was seen in section the extravasation of blood was from the portion of the vein nearest the inner surface of the mucosa. The veins of the submucosa participated in the dilatation, but were not so markedly enlarged as the veins and capillaries of the mucosa. No evidence of general giving way of the capillaries with extravasation of blood into the tissues was noted.

The small veins and capillaries of the intestines, kidney, heart, and muscles were found distended, but not nearly to the extent found in the stomach wall. No hemorrhages were observed in the heart walls or in the liver and striped muscles.

As regards fatty changes in the lining endothelium of the blood vessels, in the gastric mucosa or striped muscles, none was observed, although carefully sought for. The focal fatty changes described by Gay and Southard were not found. Neither were the "nodal" changes in peripheral nerves made out.

We find, then, that congestion and sometimes hemorrhage take place in guinea pigs dead of anaphylaxis, but these lesions are not specific. We were unable to demonstrate the fatty lesions and know, further, that they occur in other states. We are therefore unable to confirm the observations of Gay and Southard along these lines, and believe that these changes do not explain the mechanism of anaphylaxis.

PART 7.—RELATION OF TOXIC ACTION UPON GUINEA PIGS TO SERUM THERAPY.

THE RELATION OF SERUM ANAPHYLAXIS IN THE GUINEA PIG TO SERUM THERAPY.

Besredka and Steinhart[a] were the first to point out that the second injection may be given into the brain of guinea pigs. When a small quantity of horse serum is injected into the brain of a sensitized guinea pig the symptoms appear promptly and often with great violence, and death is a common result.

Besredka[b] believes that intracerebral injections may be used as a measure for the toxicity of therapeutic serums. He states that, measured in this way, different serums show a wide gamut of toxicity, the fatal dose varying from $\frac{1}{4}$ to $\frac{1}{128}$ c. c. He believes that this toxicity resides in the serum and not in the cellular elements; further, that the serums of horses living under apparently the same conditions have about the same toxicity, individual variations being rare and of little importance. He concludes that, in a general way, all serums that incite in guinea pigs grave anaphylactic phenomena in doses of $\frac{1}{16}$ to $\frac{1}{20}$ c. c. and, *a priori*, above this amount should be considered toxic.

We doubt whether there is a relation between the toxicity of serums as tested upon guinea pigs in this way and their power to produce the serum disease or collapse or sudden death in man. The unfortunate accidents, such as collapse and occasional death, depend more upon the sensitization of the individual than upon the so-called toxicity of the serum used.

Fortunately we were able to obtain two antidiphtheric serums which had been used in two cases of sudden death.

Case No. 1.—Serum No. 2277. Reported by Dr. S. N. Wiley, Norristown, Pa., Journ. Am. Med. Assn., vol. 50, Jan. 11, 1908, p. 137. Mr. E. W., aged 34 years, splendid physique, best of health. Prophylactic injection of 1,000 units antidiphtheric serum. Site of inoculation 4 inches above Poupart's ligament. Within two minutes had violent symptoms—anxious expression, itching, burning, labored breathing; lips, face, and neck swollen and red; paralysis; convulsions. Died within five minutes of injection.

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[a] Besredka and Steinhart, Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 117.

[b] Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 777.

The toxicity of this serum, i. e., another package of the same laboratory number, was tested upon the following series of guinea pigs:

TABLE NO. 38.—*Toxicity of serum No. 2277.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9147	0.23 c. c. toxine No. 7+1,420 c. c. antitoxic horse serum (PD. 09913).	97	0.1 c. c. antitoxic horse serum (2277) into brain.	Dead in 7 minutes.
9131	0.23 c. c. toxine No. 7+1,250 c. c. antitoxic horse serum (Natl. V. 24).	97	.....do...........................	Dead in 8 minutes.
9136	0.23 c. c. toxine No. 7+1,300 c. c. antitoxic horse serum (Natl. V. 24.)	97	0.05 c. c. antitoxic horse serum (2277) into brain.	Dead in 35 minutes.
9151	0.23 c. c. toxine No. 7+1,380 c. c. antitoxic horse serum (PD. 09491).	97	.....do...........................	Very severe symptoms.
9161	0.23 c. c. toxine No. 7+1,320 c. c. antitoxic horse serum (Mem. C20).	67	2 c. c. antitoxic horse serum (2277) intraperitoneally.	Dead in 3 hours.
9170	0.23 c. c. toxine No. 7+1,330 c. c. antitoxic horse serum (Mul. 2438).	67	2 c. c. antitoxic horse serum (2277) subcutaneously.	Very severe symptoms.

Case No. 2.—Serum No. 2295. Reported by Dr. H. F. Gillette, Cuba, N. Y., Journ. Am. Med. Assn., vol. 50, Jan. 4, 1908, p. 40. Mr. B., 52 years old. Had asthma and bronchial catarrh. Urine and heart normal. Rheumatic attack fifteen years prior. Coughed and raised plenty of sputum. Injection of 2,000 units antitoxic serum under left scapula. Prickling sensation in chest and neck; labored breathing; pulse regular and full. Seized with tonic spasm. Died within five minutes after injection.

The serum, i. e., another package of the same laboratory number, used in this case was tested for toxicity upon the following series of guinea pigs:

TABLE NO. 39.—*Toxicity of serum No. 2295.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9135	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V. 24).	97	0.1 c. c. antitoxic horse serum (2295) into brain.	Dead in 20 minutes.
9133	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V. 24).	97	0.05 c. c. antitoxic horse serum (2295) into brain.	Dead in 22 minutes.
9132	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Natl. V. 24).	97	.....do...........................	Dead in 11 minutes.
9373 m	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY. 306).	97	.....do...........................	Severe symptoms.
9171	0.23 c. c. toxine No. 7+1/410 c. c. antitoxic horse serum (Mul. 2438).	67	1 c. c. antitoxic horse serum (2295) subcutaneously.	Dead in 90 minutes.
9164	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mem. C23).	67	1 c. c. antitoxic horse serum (2295) intraperitoneally.	Very severe symptoms.

It has interested us very much to find that these two cases, and also others that have come to our notice, were in asthmatics. In our first publication we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man would seem to lend weight to this hypothesis. The knowledge of the fact that the injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

In order to determine the comparative toxicity of the above two serums (Nos. 2277 and 2295); we submit the following experiments showing the toxicity of serums which have been largely used in human therapy without untoward effects.

The following serums (Nos. 2364, 2369, and 2442) were kindly sent us by Dr. A. P. Hitchens, who states "he has had no report whatever concerning any untoward effect resulting from the use of either of these numbers:"

No. 2364 is antitoxic serum.

No. 2369 is antitoxic globulin.

No. 2442 is antitoxic serum.

TABLE No. 40.—*Comparative toxicity of other serums (Nos. 2442, 2369, and 2364.)*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9936	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 4 minutes.
		47	.....do.........................	Dead in 5 minutes.
9943	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Alex. 192.)	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9937	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. 192).			
9941	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2369) into brain.	Dead in 8 minutes.
9939	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Alex. 192).	47	.....do.........................	Dead in 5 minutes.
9933	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9945	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Alex. 192).	47	0.05. c. c. antitoxic horse serum (Mul. 2364) into brain.	Marked symptoms
9946	.....do.........................	47	.....do.........................	Dead in 12 minutes
9934	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2364) into brain.	Dead in 3 minutes.
9942	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	.....do.........................	Marked symptoms

The following diphtheria antitoxic horse serum (123A) was kindly sent us by Dr. William H. Park, who reports that it is of moderate strength and has given very good results in the hospital.

TABLE No. 41.—*Comparative toxicity of serum No. 123A.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9245	0.23 c. c. toxine No. 7+1/480 c. c. antitoxic horse serum (Ldrl. 11) subcutaneously.	131	0.05 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9251	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (Ldrl. 212) subcutaneously.	131	.....do.........................	Very severe symptoms.
9242	0.23 c. c. toxine No. 7+1/360 c. c. antitoxic horse serum (Mul. 2377) subcutaneously.	131	.....do.........................	Very severe symptoms.
8759	0.245 c. c. toxine No. 42+1/320 c. c. antitoxic horse serum (Strn. 1429) subcutaneously.	232	0.1 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9253	0.23 c. c. toxine No. 7+1/1500 c. c. antitoxic horse serum (Ldrl. 21B) subcutaneously.	131	.....do.........................	Dead in 10 minutes.
9239	0.23 c. c. toxine No. 7+1/800 c. c. antitoxic horse serum (Ldrl. 57) subcutaneously.	131	0.2 c. c. antitoxic horse serum (Park 123A) into brain.	Dead in 5 minutes.

The following two serums were kindly furnished by Dr. E. M. Houghton, and were extensively used in human therapy, but no complaints were received concerning them:

TABLE NO. 42.—*Comparative toxicity of serum No. 08725C.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9635	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 08725C) into the brain.	Very severe symptoms.
9644	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	......do......................	Very severe symptoms.
9645	0.23 c. c. toxine No. 7+1/660 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	......do......................	Dead in 4 minutes.
9632	0.23 c. c. toxine No. 7+1/640 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 09043C) into the brain.	Dead in 8 minutes.
9646	0.23 c. c. toxine No. 7+1/660 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	......do......................	Dead in 4 minutes.
9648	0.23 c. c. toxine No. 7+1/640 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	......do......................	Dead in 15 minutes

As a control, the following serums—some of them French antidiphtheric and some normal horse serums—are given for comparison:

TABLE NO. 43.—*Comparative toxicity of French and other serums.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
532	0.0006 c. c. tetanus toxine A+0.23 c. c. antitoxic horse serum (Ehrlich standard).	65	0.25 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
8497	0.24 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter 1828).	18	.....do......................	Very severe symptoms.
8359	0.24 c. c. toxine No. 9+1/1260 c. c. antitoxic horse serum (N. Y. 305).	35	.....do......................	Dead in 5 minutes.
9550	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (PD 07635).	62	0.2 c. c. antistreptococcic serum (Past. Inst.) into brain.	Dead in 3 minutes.
9557	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Welc. 2L839).	62	.....do......................	Marked symptoms.
9572	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. A245).	62	.....do......................	Dead in 4 minutes.
9580	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. A245).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.

Table No. 43.—*Comparative toxicity of French and other serums*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9552	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 58A).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.
9590	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245).	65	.....do........................	Dead in 4 minutes.

It therefore seems plain that the serums which do not produce untoward symptoms when injected into man, are quite as toxic upon sensitized guinea pigs, as the serums which have been followed by serious symptoms when injected into man. We believe the difference lies in the susceptibility of the individual and not in the toxicity of the serum.

PART 8.—THE EFFECT OF REPEATED INJECTIONS.

THE IMMUNIZING ACTION OF REPEATED INJECTIONS.

We found it desirable to obtain further data upon the effects of repeated injections of horse serum into guinea pigs in order to answer the question whether guinea pigs may be definitely immunized by repeated injections.

It will be seen by the following table that 10 injections of 2 c. c. of serum subcutaneously was not sufficient to render guinea pigs entirely immune. These four guinea pigs all showed mild symptoms when tested intraperitoneally fifteen or seventeen days after the last injection.

Reference to Tables No. 34, 35, 36, pages 36, 37, 38, shows the same results.

TABLE NO. 44.—*The immunizing action of repeated injections.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1371	10 injections, each 2 c. c., normal horse (roan) serum, subcutaneously, covering a period of 17 days.	15	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
1369	.....do	17	.....do	Mild symptoms.
1372	.....do	17	.....do	Mild symptoms.
1374	.....do	17	.....do	Mild symptoms.

THE SENSITIZING ACTION OF REPEATED INJECTIONS OF SMALL AMOUNTS.

The following table shows that the repeated injections of small amounts are apparently as potent in sensitizing guinea pigs as one small injection.

TABLE NO. 45.—*Sensitizing action of repeated injections of small amounts.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1364	5 injections, each 0.001 c. c., normal horse (roan) serum, subcutaneously, covering a period of 8 days.	[a]23	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 40 minutes.
1365	.....do	23	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 70 minutes.
1366	.....do	23	.....do	Dead in 75 minutes.
1367	.....do	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1368	.....do	23	0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
1363	.....do	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.

[a] From last injection.

THE EFFECT OF REPEATED SMALL INJECTIONS UPON SENSITIVE GUINEA PIGS.

We were interested in determining whether repeated small injections would immunize sensitive guinea pigs without the production of anaphylactic symptoms. For this purpose we injected a series of guinea pigs subcutaneously with normal serum in amounts too small to produce apparent symptoms and repeated the injections daily for twenty days. One series received 0.001 c. c. subcutaneously (total 0.02 c. c.) the other series 0.01 c. c. subcutaneously (total 0.2 c. c.). It will be seen from the following table that these repeated injections had little, if any, effect upon the susceptibility of these guinea pigs.

TABLE No. 46.—*Effect of repeated small injections upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9716	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Schr. II). 48 days later 20 subcutaneous injections, each 0.001 c. c., normal horse (Frank) serum daily.	[a]2	0.2 c. c. normal horse (Teddy) serum into brain.	Very severe symptoms.
9673	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do	Marked symptoms.
9678	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do	Marked symptoms.
9714	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Schr. II). 48 days later 20 injections 0.01 c. c. daily.	2	.....do	Dead in 5 minutes.
9715	.....do	2	.....do	Marked symptoms.
9677	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.01 c. c. daily.	2	.....do	Marked symptoms.
9155	0.23 c. c. toxine No. 7+1/200 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 120 minutes.
9156	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.2 c. c. normal horse (roan) serum into brain.	Dead in 7 minutes.
9252	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 90 minutes.

[a] After last injection.

TABLE No. 46.—*Effect of repeated small injections upon sensitive guinea pigs*—Con.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9186	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Mul. 2362). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
9157	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Slight symptoms.

ATTEMPTS TO IMMUNIZE SENSITIVE GUINEA PIGS BY REPEATED INJECTIONS OF HEATED SERUM.

In the following series sensitized guinea pigs were injected with 1 c. c. of normal horse serum heated to 100° C. for one hour. The serum was first diluted in the proportion of 1 part to 3 of water to prevent coagulation. The 1 c. c. of the mixture injected into the guinea pigs represents, therefore, 0.25 c. c. of horse serum. The pigs received from 20 to 25 injections subcutaneously. They therefore received a total amount of 5 to 6 c. c. of normal horse serum. It is evident from the table that these injections failed to materially modify the susceptibility of the guinea pigs.

TABLE No. 47.—*Effect of heated serum upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9197	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 62 days later 20 injections 1 c. c. daily normal horse (roan) serum heated 100° C. 1 hour, subcutaneously.	[a]10	0.2 c. c. normal horse (roan) serum into brain.	Dead in 10 minutes
9179	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2443). 62 days later 22 injections 1 c. c. daily heated serum.	8	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
9180	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Mul. 2443). 62 days later 25 injections 1 c. c. daily heated serum.	4	6 c. c. normal horse (roan) serum subcutaneously.	Marked symptoms.
9191	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mul. 2358). 62 days later 24 injections 1 c. c. daily heated serum.	6	.....do.........................	Very severe symptoms.

[a] After last injection.

PART 9.—THE RELATION OF ANAPHYLAXIS TO THE TOXEMIAS OF PREGNANCY.

The symptoms which cause puerperal eclampsia and the conditions under which it occurs suggest that anaphylaxis may explain some of the mystery of this state.

It occurred to us that either the blood or proteid substances in solution from the fetus or the placenta may first sensitize the mother. A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and the symptoms which occur in a certain class of the toxemias of pregnancy.

"Through the establishment of the pathological anatomy of the condition a general agreement has been reached that puerperal eclampsia must be included among the diseases caused by toxic materials of unknown origin and nature."[a] A certain class of the toxemias of pregnancy are sometimes spoken of as reflex or neurotic origin.

There seems to be a fair agreement that the placenta must be the source of toxic material, especially as typical cases of eclampsia and pernicious vomiting have been observed in patients with hydatid mole, in which cases, of course, toxic matter of fetal origin could be eliminated. Furthermore, eclampsia may appear after the fetus has been removed. Much attention was therefore given to the hypothesis elaborated about four years ago by Veit, Weichardt, and others that through the entrance of placental cells into the circulation of the mother an intoxication was caused either by disintegration of the cells and the formation of toxic substances or in the development of antisubstances by the maternal organism.

In spite of much experimentation and discussion, however, no satisfactory conclusions have yet been reached concerning the validity of this hypothesis, and Martin has secured some very valuable evidence that at least in rabbits entrance of their own placental elements into the circulation in large amounts does not cause any serious disturbance. So far as we are aware, we are the first to suggest that certain of the toxemias of pregnancy may be a condition of hypersusceptibility.

Along these lines we first made a number of experiments to determine whether the fetal blood of the guinea pig could sensitize the mother guinea pig. We injected a number of female guinea pigs, both pregnant and not pregnant, with fetal blood, and after an appropriate interval gave them a second injection of the same material.

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[a] From a recent discussion of the theories concerning the causes of the toxemias of pregnancy. Editorial in the Journal of the American Medical Association, vol. 50, No. 2, Jan. 11, 1908, p. 124.

All these experiments resulted negatively, which was anticipated from our previous studies upon the effect of homologous blood serums. This tends to confirm the clinical observations that the poisons causing the toxemias of pregnancy do not come from the fetus.

We then made a series of experiments upon female guinea pigs with placental extracts. The placenta (almost at full term) was ground up in a mortar and allowed to "autolyze" about an hour at room temperature, and some of the resulting extract was injected subcutaneously into female guinea pigs.

After an interval of twenty-two days the guinea pigs were again inoculated with a placental extract. This time the placenta was allowed to "autolyze" three hours in the incubator (37° C.). Five pigs were tested with this placental extract; three of them were given 6 c. c. into the peritoneum, two of these three showing pronounced symptoms of anaphylaxis. The remaining one showed slight symptoms. Six cubic centimeters of the same placental extract injected into the peritoneal cavity of two young normal guinea pigs as a control produced no apparent effect. The remaining pigs were injected with small quantities of the extract intracerebrally, with negative results.

These experiments were repeated with precisely similar results. Thus four more guinea pigs were sensitized with different quantities of guinea-pig placental extract, and after a period of twenty-two days were given a second injection of similar placental extract. The extract used at both the first and second injections in these four pigs was autolyzed three hours in the incubator, then strained through gauze. All four of them showed definite symptoms.

In a third experiment three guinea pigs were sensitized subcutaneously with placental extract (three hours at 37°), and after an interval of twenty-four days the second injection was given directly into the circulation by the intracardiac method. These three pigs showed severe and early symptoms, including convulsions. Three normal control guinea pigs treated in the same manner did not respond. The three pigs that responded to the second intracardiac injection were autopsied and found to have a fresh current-jelly clot in the pericardial sac. How much this hemorrhage, which is probably due to the puncture of superficial vessels of the heart, may account for the symptoms is doubtful. Further work along this line is in progress.

From this limited series it is evident that the mother guinea pig may be sensitized with the autolytic products of her own placenta. These experiments naturally suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea pig which we are studying. Further studies along this line are now being made, especially to determine whether the liver lesions may thus be produced in the guinea pig and other animals.

PART 10.—MISCELLANY.

TIME.

The longest time that has elapsed between the first and the second injections in a guinea pig in our experiments has been two years two days (732 days). It therefore seems that guinea pigs once sensitized with mixtures of antitoxic horse serum and diphtheria toxine are sensitive throughout practically the remainder of their lives.

Time.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
4523	0.19 c. c. toxine No. 7+1 immunity unit antitoxic horse serum (B27).	732	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 60 minutes.

TABLE No. 48.—*Clam juice.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1650	0.1 c. c. clam extract subcutaneously.	127	6 c. c. clam extract subcutaneously.	Marked symptoms.
1651	.....do	127	.....do	Do.
1652	.....do	127	6 c. c. clam extract intraperitoneally.	Dead in 50 minutes.
1653	.....do	127	.....do	Marked symptoms.
1654	.....do	127	.....do	Do.

The above series shows that the protein matter of clams may sensitize and poison guinea pigs.

PART II.—SUMMARY AND CONCLUSIONS.

The *period of incubation* of serum anaphylaxis is about seven days in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. It also appears that the sensitization comes on somewhat gradually.

Judged by our results and the work of others, the period of incubation is quite constant.

It seems that the period of incubation is not appreciably prolonged by a large sensitizing dose.

Animals sensitized with horse serum alone remain so for a long period of time (at least 245 days). Guinea pigs sensitized with the toxine-antitoxin mixture remain sensitive throughout the remainder of their life (at least 732 days).

The *sensitizing principle* is gradually influenced by heat. It disappears almost entirely when horse serum is heated to 100° C. for one hour.

Guinea pigs may be sensitized by intracerebral injections, provided quantities of 0.000,1 c. c. or more are used. We obtained negative results with sensitizing doses of 0.000,01 c. c. into the brain.

Guinea pigs may be sensitized by dropping horse serum upon the eye.

The *toxic principle* in horse serum is gradually destroyed by heat.

A temperature of 70° C. for one hour does not seem appreciably to diminish the poisonous property of horse serum, but it seems to be affected at 80° C. for one hour. At 90° C. for one hour it still remains slightly toxic, but at 100° C. for one hour the toxicity apparently disappears.

The difference in the effect of heat upon the sensitizing and the toxic principle may be more apparent than real, for exceedingly minute amounts of serum will sensitize guinea pigs, while it would take a very large quantity of weakened serum to produce symptoms at the second injection.

The toxicity of horse serum does not appear to diminish with the age of the serum.

No favorable influence upon the anaphylactic state was obtained by injecting pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chloride, and calcium acetate into guinea pigs the day before they were tested.

Iodine also apparently had no modifying effect upon serum anaphylaxis, whether dissolved in the serum or injected separately into the guinea pig.

Methemaglobin-producing substances, such as the nitrites, do not hinder anaphylaxis.

Ether narcosis masks the symptoms, but does not prevent the fatal issue of a second injection.

Further attempts to find free antibodies to neutralize the toxic action of horse serum by treating it with the sensitized guinea pig serum, and also with the brain substance of sensitized guinea pigs, proved negative.

The *specific nature of anaphylaxis* is further shown by various experiments. For example, guinea pigs sensitized with three separate proteins, viz, horse serum, egg white, and cow's milk, contain three separate anaphylactins in their blood.

Guinea pigs sensitized with human milk do not react to a second injection of cow's milk. This again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter of human and cow's milk.

Guinea pigs sensitized with sheep's milk react to a subsequent injection of cow's milk.

Guinea pigs sensitized with dog's milk do not react to a subsequent injection of cow's milk.

Guinea pigs sensitized with hen egg white react to a subsequent injection of duck egg white; and guinea pigs sensitized with duck egg white react to a subsequent injection of hen egg white. The results of similar studies with goose, guinea hen, crane, pigeon, and turkey egg albumin are recorded in the text.

The anaphylactic reaction in the guinea pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species or between proteins of widely different origin.

A substance known as "*anaphylactin*" is present in the blood serum of sensitized guinea pigs. This substance is not present during the period of incubation.

We have been unable to demonstrate the presence of anaphylactin in the blood serum of man, the monkey, and the cat.

Anaphylactin is present in the blood serum of immune guinea pigs.

The mechanism of anaphylaxis.—We find that congestion and sometimes hemorrhages may be present in guinea pigs dead of anaphylaxis, but these lesions are not always apparent, and, furthermore, are not specific.

We were unable to demonstrate fatty lesions in guinea pigs dead of anaphylaxis, and know, further, they occur in other states.

We believe that these morphological alterations do not explain the mechanism of anaphylaxis. It is probable that the mechanism will not be unraveled until further light is shed upon the chemistry of protein metabolism.

Cases of sudden death in man.—Our experiments demonstrate that the horse serum used in cases followed by sudden death is no more toxic for guinea pigs than antitoxic horse serums used extensively in human therapy without untoward symptoms.

It is our belief that it is not the special toxicity of the horse serum, but the sensitization of the patient, which accounts for the collapse or sudden death sometimes following the injection of horse serum.

We are still unable to account for the ways in which man may be sensitized to a foreign protein. It seems perfectly plain, however, that man may be so sensitized.

In previous publications we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man seems to lend some weight to this hypothesis.

The knowledge of the fact that an injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

The *repeated injections* of small amounts of horse serum sensitizes guinea pigs.

Repeated injections of large amounts render guinea pigs partially immune.

Repeated injections of small amounts of serum into sensitized guinea pigs have no appreciable effect.

Sensitized guinea pigs can not be immunized by repeated injections of heated serum (100° C. for one hour).

We suggest a possible relation between the *toxemias of pregnancy and anaphylaxis.*

Guinea pigs can not be sensitized with guinea-pig fetal blood. This shows that the fetal blood of the guinea pig does not contain an alien protein for the mother.

Guinea pigs may be sensitized and subsequently poisoned with guinea-pig placental extracts.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 46
JUNE, 1908

HEPATOZOON PERNICIOSUM (N. G., N. SP.); A HAEMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (*LELAPS ECHIDNINUS*)

BY

W. W. MILLER

WASHINGTON
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1908

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SUMMARY.

Hepatozoon perniciosum, n. g., n. sp., is a hæmogregarine found in white rats in Washington, D. C., and the cause of an epizootic observed among these animals. The protozoon is conveyed by a mite (*Lelaps echidninus* Berlese), which is the true intermediate host. Infection is transmitted to the rat when the mite is swallowed by the rat.

The mites are ecto-parasites upon the rats, from which they receive infection by sucking the blood.

Multiplication of the hæmogregarine in the rat takes place in the liver. In the stomach of the mite the hæmogregarines conjugate and form an ookinet, which penetrates the stomach wall and completes its development in the body tissues of the mite.

The illustrations in this bulletin have been prepared from drawings made by the laboratory artist, Prof. Leonard H. Wilder.

HEPATOZOON PERNICIOSUM (N. G., N. SP.)—A HÆMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (LELAPS ECHIDNINUS BERLESE).[a]

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Washington, D. C.

INTRODUCTION.

Although the first of the Hæmocytozoa to be discovered (*Lankesterella ranarum,* by Lankester in 1871) was a hæmogregarine, our knowledge to-day of this important group is far from complete. A great number of hæmatozoa have been described in cold-blooded animals, in nearly all of which there is a more or less close resemblance to the gregarine type. The most noteworthy peculiarity of these parasites is the wormlike form assumed during at least some portion of the life cycle. They are, at some period, endoglobular; but they lack the ameboid motion, and pigmentation so characteristic of the hæmamœbæ. Multiplication occurs by division into two or into many individuals.

Classifying them according to the vertebrate host, the following is a brief description of the known species.

HÆMOGREGARINIDÆ OF AMPHIBIA.

About ten species of hæmogregarines in batrachians are known, all of which affect the red blood cells of frogs and toads. Until quite recently the belief was unquestioned that sexual as well as nonsexual multiplication occurred in the same host. In fact, Hintze[b] interpreted certain appearances as a conjugation of the parasites and the formation of an ookinet which entered the intestinal epithelium, became encysted, was cast off in the feces and served, when ingested by another frog, to propagate the parasite.

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[a] Manuscript submitted for publication June 25, 1908.

[b] Hintze, R.: Lebensweise und Entwicklung von *Lankesterella minima* (Chaussat). Zoolog. Jahrb., Abt. f. Anat. u. Ontog., Bd. 15, Heft 4, 1904, pp. 693–730.

Lühe believes this observation to be in error and regards the oocyst as coccidial in nature. The multiple infections of protozoa (flagellates, coccidia, and hæmogregarines) found in frogs, often in the same animal, renders it very difficult to determine positively to which parasite certain phases in the life cycle belong.

A recent observation by Durham[a] upon the life history of *Drepanidium* in a South American toad is of special interest. He found that nearly all the toads were infested with a species of tick. In the stomach of the tick were seen forms of the *Drepanidium* apparently in copulation, also peculiar cysts 60 micra in diameter, which he believed to be some stage in the life cycle of the parasite. Durham regards the tick as the probable intermediate host, although this is not definitely established.

The studies of Billet[b] and also some recent observations of Laveran and Nègre[c] seem to indicate that trypanosomes may play a part in the life cycle of *Lankesterella* and other hæmogregarines. Beyond question, a great deal of work is necessary upon this group before many doubtful points can be clarified.

HÆMOGREGARINIDÆ OF SNAKES AND LIZARDS.

Lühe[d] mentions nineteen species affecting the red blood corpuscles of snakes and lizards. He divides them into two genera, *Karyolysus* and *Hæmogregarina*. A noteworthy peculiarity of the former is the existence of large and small trophozoites (without intermediate stages)—the macro-merozoites and micro-merozoites. Schizogony takes place in red blood cells in the capillaries of internal organs. The membrane remaining after destruction of the red cell forms a sort of a cyst wall. The schizonts are of two types, macro-schizonts and micro-schizonts, corresponding to the two varieties of merozoites.

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[a] Durham, H. E.: *Drepandium* in the toad. Liverpool School of Tropical Medicine, Memoir VII—Report of the yellow-fever expedition to Para, Liverpool, 1902, pp. 78–79.

[b] Billet, A.: Sur le *Trypanosoma inopinatum* de la grenouille verte de l'Algérie et sa relation possible avec les *Drepanidium*. C. R. Soc. Biol., Paris, vol. 57, No. 27, 1904, pp. 161–165.

[c] Laveran, A., & Nègre: Sur un protozoaire parasite de *Hyalomma ægyptium*. C. R. Soc. Biol., Paris, vol. 58, no. 21, 1905, pp. 964–966.

[d] Lühe, M.: In Handbuch der Tropenkrankheiten, C. Mense, Leipzig, 1906, Bd. III, pp. 209–210.

Lutz[a] regards the small forms as male and the large as female. He has never seen evidence of conjugation of the trophozoites in the blood of the host. The intermediate host is unknown.

HÆMOGREGARINIDÆ OF TURTLES AND CROCODILES.

A large number of hæmogregarines have been described in turtles. *Hæmogregarina stepanovi* Danielewsky has been carefully studied by Danielewsky, and in its sexual development by Siegel.[b]

In *Hæmogregarina stepanovi* the gametocytes, according to Siegel, are the elongated trophozoites which are bent into a U shape on account of their length being greater than that of the red blood cell which they inhabit. Schizogony occurs as in other hæmogregarines. There is no dimorphism of the schizonts. The invertebrate host is a leech, in the gut of which the gametocytes develop and fertilization takes place. The ookinet which develops from the zygote invades the intestinal wall and enters the esophageal glands, where elongated sporozoites are formed. Infection of the turtle, to which the leech next attaches itself, is said to take place in a manner similar to the malarial infection from mosquitoes. Siegel believes that the ovary and eggs of the leech may be invaded by the sporozoites, and the next generation of leeches thus infected.

HÆMOGREGARINIDÆ OF FISHES.

About twelve species are known. *Hæmogregarina bigemina*, from the blood of blennies (*Blennius pholis*), has been described by Laveran and Mesnil.[c] In the nonsexual multiplication of this parasite by binary fission a resemblance to the manner of multiplication in piroplasma is observed, and incidentally to that in trypanosomes. In this connection it is interesting to note that trypanosomes have been found very frequently in the blood of fishes, which also harbor hæmogregarines. The group is as yet imperfectly known. The mode of transmission of the infection has not been determined.

In all hæmogregarines of cold-blooded animals which have been studied, nonsexual multiplication or schizogony takes place in the red blood corpuscles, usually in some internal organ. The merozoites, when set free, invade other red blood cells, and increase considerably in size before reaching maturity.

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[a] Lutz, A.: Ueber die *Drepanidien* der Schlangen. Ein Beitrag zur Kenntniss des Hamosporidien. Centblt. f. Bakt., 1. Abt., vol. 29, 1901, pp. 390–398.

[b] Siegel: Die geschlechtliche Entwicklung von *Hæmogregarina stepanovi* in Rüsselegel *Placobdella catenigera*. Arch. f. Protistenkde., 1903, Bd. 2, H. 3, pp. 339–342.

[c] Laveran, A., & Mesnil, F.: Deux hémogrégarines nouvelles des poissons. C. R. de l'Acad. des Sci., vol. 133, 1901, pp. 572–577.

Although the first protozoan blood parasites to be observed, with the exception of trypanosomes, were hæmogregarines, and since that time a great number have been discovered in cold-blooded animals and more or less completely described, knowledge of such forms in warm-blooded animals dates back only three or four years.

In 1905 James[a] published a description of a new parasite found in the white blood cells of pariah dogs in India, their occurrence having been previously noted by Bentley.[b] James did not regard the parasite, which he named *Leucocytozoon canis,* as a hæmogregarine, but considered its relationship to parasites in the blood of birds, certain forms of which appear to infect the white blood corpuscles.

These so-called "leucocytozoa" were first described by Danielewsky[c] in the blood of owls.

Quite recently Schaudinn,[d] Laveran,[e] and Berestneff[f] have shown that the forms of this parasite (now known as *Leucocytozoon ziemanni*), which resemble parasites in leucocytes, are in reality macrogametes to which are attached portions of the nucleus of the red cell originally invaded. The name is somewhat misleading.

James found *Leucocytozoon canis* present in the blood of 6 out of 45 dogs examined. The polymorphonuclear cells were the only variety affected. The largest percentage of infected corpuscles recorded was 9 per cent. In one single instance only was a parasite found free in a stained blood smear. On one occasion a free form was seen in a fresh preparation, having escaped from the leucocyte by reason of its continuous vermicular movements. The wormlike body remained in contact with the host cell, but continued its movements for an hour or more.

The intracorpuscular forms, when stained by Romanowsky's method, are described as oblong bean-shaped bodies enveloped by the protoplasm of the leucocyte and apparently surrounded by a delicate cyst. In addition to the bean-shaped bodies, James describes parasites, almost round in shape, with a very indistinct capsule. The leucocytes affected are of the polymorphonuclear variety. The nucleus in 86 per cent of the parasites is situated near one extremity and is oval or round in shape, and stains like the nuclei of the leucocytes.

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[a] James: On a parasite found in the white corpuscles of the blood of dogs. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1905, n. s., no. 14.

[b] Bentley: Preliminary note upon a leucocytozoon of the dog. Brit. med. journ., May 6, 1905, p. 988.

[c] Danielewsky, B.: La parasitologie comparée du sang. Kharkoff, 1889. Also, Developpement des parasites malariques dans les leucocytes des oiseaux. Ann. de l'Inst. Pasteur, 1890, p. 427.

[d] Schaudinn, F.: Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochæte.* Arb. a. d. Kaiserl. Gesundheitsamte, vol. 20, 1904, p. 387–439.

[e] Laveran, A.: Contribution à l'étude de *Hæmamœba ziemanni.* C. R. Soc. Biol., vol. 55, 1903, p. 620–623.

[f] Berestneff, N.: Ueber das Leucocytozoön Danielewsky. Arch. f. Protistenkde, vol. 3, 1904, p. 376–386.

The manner in which nonsexual multiplication takes place in the bone marrow is described by Christophers.[a]

Since the discovery of *Leucocytozoon canis* by Bentley a number of somewhat similar parasites have been observed in the blood of mammals, all in tropical regions. In some instances they affect only the white corpuscles, in others only the red cells, but never both. Certain peculiarities and their similarity to hæmogregarines in cold-blooded animals are sufficient to identify them in the Hæmogregarinidæ.

In 1905 Balfour[b] described a new parasite in the red blood cells of *Jaculus jaculus*, the jerboa, or kangaroo rat, of Sudan. This organism, according to Laveran, is a hæmogregarine.

The trophozoite, which is either free or lies in the remains of a red blood cell, is sausage shaped, nonpigmented, and nonmotile. It measures 5.5 to 7 micra in length and from 1.4 to 2.8 micra in breadth. When stained by the Leishmann-Romanowsky method, the nucleus is large, oval, and centrally located. In the peripheral blood all forms are in about the same stage of development. The animal host does not suffer in health.

The parasite (*Haemogregarina balfouri*) of the jerboa is bent slightly upon itself and its length is a little greater than that of the red corpuscles. In addition to the form described, Balfour observed on one occasion a free motile vermicule form without flagellum. In a stained specimen which showed this stage the total length was 15.5 micra, one extremity being finely tapered to a point, the other rounded and containing chromatin granules. Multiplication occurs by schizogony. The schizonts are found in the liver, kidney, and bone marrow, in the form of cysts. The cyst walls appear to be formed of the remnant of the cell of the organ invaded. In the liver the schizonts appear quite similar to those of certain coccidia. The manner of invasion of the liver cells has not been determined. No sexual cycle or mode of transmission is known. Balfour states that no naked-eye indications of disease are present in an infected animal. The spleen is not enlarged. He also mentions the fact that he has discovered what seems to be the same parasite (*H. balfouri*) in the mononuclear leucocytes of a rat (*Mus decumanus*) in Khartoum.

In 1905 Christophers[c] discovered a parasite in the red blood cells of the Indian field rat (*Gerbillus indicus*) which resembles *H. balfouri*. The affected corpuscles (somewhat enlarged) contained a minute motionless vermicule with a short, sharply flexed tail (trophozoite). A distinct space surrounded the parasite. In some fresh preparations there were free parasites similar to the above, except that the pointed extremity was no longer bent. They exhibited active gliding movements, also twisting and turning motions. In stained specimens the trophozoites were in the red cells and appeared to be enveloped by a capsule. A large chromatin body was situated in the "tail" of the parasite, also occasional scattered granules.

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[a] Christophers, S. R.: *Leucocytozoon canis*. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 26.

[b] Balfour, A.: A hæmogregarine of mammals. Journ. Trop. Med., vol. 8, 1905, p. 241; vol. 9, 1906, pp. 81–84.

[c] Christophers, S. R.: *Hæmogregarina gerbilli*. Sci. Mem. Officers Med. & San. Dept. Gov. India, 1905, n. s., no. 18.

No certain sexual differentiation of the trophozoites could be made out. Although all the organs of infected rats were carefully studied, no evidence of nonsexual multiplication could be found. Infected rats showed no constitutional disturbances. Differential leucocyte counts indicated that the percentage of different kinds of leucocytes differed but little from the normal.

The only ecto-parasites found upon the rats were insects—a new species of louse (*Hæmatopinus*) measuring 1.4 by .5 mm. Certain structures were encountered in the dissection of these lice which Christophers regarded as phases of the sexual cycle of *H. gerbilli*. Although experiments were made to infect lice from infected animals, no recorded attempts were made to transmit the infection from one animal to another by means of lice. In his experiments a number of lice were placed upon a young heavily infected rat. These were removed at stated intervals and examined. On the fourth day two lice were removed and examined. In the midgut and intestine vermicules in active motion were observed. On the seventh day large cysts were observed lying free in the body cavity of the insect. They were of large size, measuring as much as 350 micra in diameter. When dissected out they appeared as minute white spheres which, with the microscope, were observed to be filled with minute cysts containing six or eight crescentic bodies. Younger cysts measuring 10 micra in diameter were also seen, also intermediate ones varying in size between the smallest and the largest. No satisfactory stained specimens of these intermediate stages were obtained. No abnormal appearance was observed in the salivary glands of any of the lice examined. When ruptured cysts were mixed with the blood of the Indian rat and examined with the microscope, the crescents became active vermicules.

No forms suggesting sexual elements or conjugation were observed by Christophers. In a recent work this writer[a] states, regarding the life cycle of *H. gerbilli* in *Hæmatopinus stephensi:* "Vermicules are freely liberated in the midgut and in the body cavity large cysts are found; but, as a result of work on *L. canis* in the tick, I am doubtful whether the cysts have anything to do with the hæmogregarine."

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[a] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

Patton [a] has described a parasite in the large mononuclear leucocytes of palm squirrels. Forms measuring 10 micra in length were seen, one extremity being bent and somewhat pointed, resembling a tail. Free vermicules exhibited slow "streaming" movements. Patton made a number of leucocyte counts upon blood from infected and noninfected squirrels and found both a relative and numerical increase of the large mononuclears in the former. Smears were made from all the organs of the squirrel, but no evidence of multiplication was observed.

Lice (a species of *Hæmatopinus*) were found upon many of the squirrels examined. Upon dissection active vermicules measuring 15.5 micra were seen in the gut of the lice which had been placed upon infected squirrels. No further development was observed, nor were any parasitic bodies found in the ovaries or salivary glands.

Christophers [b] has recently published his observations upon the sexual cycle of *Leucocytozoon canis* in the tick. He had previously described the schizogony, which takes place in the bone marrow, but never in the liver or kidney. The merozoites are formed in cysts. One cyst may contain 30 or more merozoites.

The sexual cycle in the dog tick (*Rhipicephalus sanguineus*) is described as follows: In the gut of ticks examined immediately after removal from infected dogs free vermicules are seen; after twenty-four hours they have penetrated the cells of the gut wall and are seen in various stages of fission, which precedes the formation of the sexual elements. On the third day conjugation bodies and the first stages of the development of the fertilized oocyst are seen. Mature oocysts are observed on the fourth day. According to Christophers, the vermicules, after entering the intestinal cell, divide into two, four, or even eight vermicules. Two of these vermicules then conjugate and an oocyst is formed, which, when fully developed, measures 14 micra in diameter and splits up into 12 to 14 sporozoites, without an investing capsule. The entire process takes place in the epithelium of the gut wall and not in the cœlomic cavity of the tick.

From the description of Christophers it seems difficult to understand his interpretation of some of the stages observed. Especially it would seem difficult to distinguish the parasites formed by fission of the vermicules from the free sporozoites. He mentions no feature which distinguishes the male elements (microgametes) from the female (macrogametes).

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[a] Patton, W. S.: On a parasite found in the white corpuscles of the blood of palm squirrels. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 24.

[b] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

The method of inoculation of a dog with *L. canis* by the tick is unknown. Christophers states that since the female tick does not suck blood again after repletion, the passage of the sporozoite into the ova seems a necessity if the parasite is to regain entrance into the mammalian host. He has been unable to find sporozoites in the ova or, in fact, anywhere except in the gut of the ticks.

Adie,[a] in India, has observed in blood smears from *Mus rattus*, in each instance accompanied by trypanosomes, a leucocytozoon with an oval nucleus and without cytocyst, and about one-third the length of the leucocyte in which it was inclosed. Further details regarding it are lacking.

Summary.—A critical examination of the literature dealing with the Hæmogregarinidæ reveals the fact that almost nothing is definitely known concerning their sexual cycle and manner of transmission.

HEPATOZOON PERNICIOSUM.

MATERIAL AND TECHNIQUE.

An examination of a blood smear from a white rat which had died from an unknown cause revealed a large number of protozoan parasites somewhat resembling *Leucocytozoon canis*. It was found upon inquiry that the stock of white rats kept for laboratory use, in cages, in a wooden building adjoining the stable was being greatly depleted by disease. Many of the animals became anæmic and emaciated and after a longer or shorter period, usually a week or more, died. Upon examination it was found that some of the cages were infested with mites, which were present in large numbers upon the rats. Some of these mites, taken from an infected rat, were found to harbor the leucocytic parasite found in the blood of the rats. A more extended study revealed the interesting fact that the sexual phase of the parasite occurs in the mite.

Seventeen rats, four of which died later, were made the basis of a study of the parasite in the rat. In addition, a large amount of material was obtained from rats experimentally infected. Material in a perfectly fresh condition was taken from all organs and preserved in fixing fluids. All the customary fixatives were employed, but the most satisfactory reagent for preserving the parasites in tissues was found to be formalin (formalin = 40 per cent formaldehyde). In strengths of 4 to 10 per cent of formalin in normal salt solution the tissues were immersed for twenty-four hours, then washed out in water and preserved in 70 to 80 per cent alcohol. Sections varying in thickness from 5 to 15 micra were made from paraffin-embedded tissue. The stains used for sections were Weigert's iron hematoxylin, Delafield's hematoxylin, and Giemsa's solution for Romanowsky staining. Smears from blood and the various organs were prepared, both dry and fixed in alcohol, formol alcohol, and methyl alcohol. Giemsa's and Wright's stains were found the most satisfactory for blood smears.

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[a] Adie, J. R.: Note on a leucocytozoon found in *Mus rattus* in the Punjaub. Journ. trop. med., vol. 9, 1906, p. 325

In drawing blood from living rats some difficulty is usually experienced. If a portion of the tail (about 1½ inches) is removed, sufficient blood for smears is readily obtained. If the blood examination is to be repeated daily, a slight manipulation causes the granulation tissue formed upon the stump to bleed freely. The increased number of leucocytes on account of the slight inflammatory reaction from the wound does not interfere with the detection of the parasite. Obviously blood obtained in this manner could not be used for differential or total counts of the white blood cells, in which instance it is necessary to remove another small piece from the extremity.

In handling the rats a pair of long curved surgical dressing forceps, with which the animal is grasped by the loose skin at the back of the neck, are very convenient.

Hepatozoon perniciosum in the Blood of White Rats.

When stained smears made from the blood of infected rats are examined with the microscope, large oval nucleated bodies are seen embedded in the protoplasm of leucocytes. The white cells affected are almost invariably large mononuclear lymphocytes, seldom transitional leucocytes, and very rarely polymorphonuclear leucocytes. The mononuclear and transitional cells of the rat's blood resemble very closely cells of the same type in human blood. The nuclei stain strongly with basic stains, and the protoplasm is free of basic staining granules. The nuclei of the former variety are almost always excentrically located in the cell protoplasm, and the parasite, when present, is usually situated beside the nucleus, where the protoplasm is most abundant. In transitional cells the parasite is often wedged in the angle formed by the bending of the nucleus. The white cells which contain parasites appear to suffer in no way by reason of their presence. Evidence of degenerative or other changes is lacking.

A marked increase in the number of leucocytes, especially of the large mononuclear variety, is invariably observed when parasites are present. This increase varies in direct proportion to the number of parasites in the blood. The blood in severe infections, shortly before death, strongly suggests that seen in human lymphatic leukemia, both in the fresh and stained condition. Nucleated red cells, which are present in small numbers in normal rat's blood, are often greatly increased in numbers. A series of actual and differential leucocyte counts of the blood of rats, with infections of varying severity, is given below.

Rat No.	Large lymphocytes containing parasites.	Number of parasites free.	Total white blood cells.	Large mononuclears.	Transitional.	Polymorphonuclears.	Eosinophiles.	Small mononuclears.
	Per cent.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	72.	2	36,000	62.	6.2	20.9	1.1	9.8
2	16	4	32,600	48	5.5	31.1	2.1	8.3
3	53.5	6	102,000	65.1	9.6	18.3	.8	7.2
4	30	0	16,000	51.3	4.5	31.2	.6	12.4
5	82.5	6	128,000	78.7	6.5	11.6	.3	2.9
6	39	0	28,500	45.8	6	39.6	.5	8.1
7	48	1	64,000	50.1	8.6	35.5	1.8	4
8	2	0	12,600	30.2	8.5	51	2	8.3
9	12	0	10,800	35.6	10.5	48	1.5	4.4
10	20.5	0	15,200	30.5	7	50.7	1.6	10.2

A number of red blood cell counts made upon the blood of heavily infected rats gave from 1,900,000 to 3,200,000 per cubic millimeter. Hemoglobin, with the Tallquist scale, from 20 to 60 per cent.

PARASITES IN FRESH BLOOD.

When a drop of blood is spread out thinly between cover slip and slide the parasites are readily made out in the leucocytes. They appear as large rod-shaped bodies with rounded extremities and are slightly more refractile to light than the leucocytes. No nucleus can be detected. In the fresh condition the parasite appears longer and more slender than when stained. It is often longer than the leucocyte in which it is contained, the protoplasm of the latter appearing to be stretched somewhat in order to encompass the parasite.

The first studies made upon *H. perniciosum* in the blood were limited to material obtained from naturally infected rats, in all of which the infection had existed for some time before the discovery of the microorganism. In the blood of these rats nearly all the parasites were in leucocytes, and very few were observed free. In rats artificially infected, and in which daily blood examinations were made from the beginning, conditions were found to be somewhat different at the inception of the disease.

The first parasites observed in recently infected animals were free in the blood plasma, few in number, and actively motile. Later, the common encysted forms appeared and the motile forms—the ''vermicules''—disappeared. In a few rats, vermicules were found during the course of the disease and especially just before death. The vermicules, as observed, are slender bodies 15 to 16 micra in length, with clear delicately striated nongranular protoplasm, somewhat refractile to light. The anterior extremity, bluntly pointed, is always the one directed forward when in motion. The posterior extremity is narrower, rounded, and nearer the nucleus. The movements, size, and appearance of the vermicules are identical with those of vermicules formed from sporozoites, which will be described later in detail.

PARASITES STAINED.

In blood smears stained by Giemsa's or Wright's stain the parasites appear as elongated oval bodies, broader and shorter than in the fresh blood, measuring on the average 12 by 6 micra. The nucleus stains almost the same color as the nucleus of the leucocyte in which it appears. The protoplasm of the parasite stains pale blue (more strongly at the periphery), is uniform in character, and is free of granules. The nucleus usually occupies the middle third of the parasite; occasionally it is located at the extremity. In outline the common form is somewhat quadrilateral with rounded corners or is irregularly oval. Instead of being made up of chromatin granules the nucleus is composed of skeins or bands of chromatin, often arranged in a more or less parallel fashion at right angles to the parasite. It is probable that the nucleus is quite thin and flat, since parasites are frequently seen in which the chromatin is arranged in a single narrow band near and parallel to one side. Surrounding each parasite and separating it from the protoplasm of the leucocyte is a delicate cyst wall, which appears usually as a clear, unstained, narrow area of even thickness. The superficial layer of protoplasm of the parasite seems condensed. That the cyst is an integral part of the latter and not of the leucocyte is shown by the fact that free encysted forms are occasionally seen.

The behavior of the parasites toward stains is an additional evidence of the presence of a cyst wall. When less powerful stains are employed than those mentioned the parasite stains with great difficulty, although the leucocytes are strongly stained. In faintly stained specimens the parasites appear as colorless, refractile bodies, the interior being unstained.

It is noteworthy that in *H. perniciosum*, as in other hæmogregarines of mammals, the forms met with in the circulation—the trophozoites—are approximately equal in size. Moreover, the dimensions do not vary greatly in different animals or in the same animal at different times. This peculiarity is explained, as will be shown later, by the fact that the parasites are discharged only when fully grown into the circulating blood.

In stained specimens the free vermicules are slender, curved or straight, with blue staining protoplasm, devoid of granules and without a capsule. The nucleus is formed of several rounded chromatic bodies (pl. II). One extremity is often pointed, and the vermicule is longer and slenderer than the encysted parasite. The latter unquestionably originates from the vermicule and all gradations between the two are observed, especially in smears from the liver.

In the first stage the vermicule becomes broader and shorter, retaining the granular nucleus; next, the latter is changed to the bandlike or homogeneous form, either before or after being engulfed by a phagocytic mononuclear or transitional white cell; and finally a capsule is developed. The ultimate fate of the parasites is uncertain. In rats with a mild grade of infection the encysted trophozoites may be found in moderate numbers six to eight weeks or even longer after the onset of the disease; yet when the livers of such animals are examined no schizonts are found. From this it is evident that the trophozoites after becoming encysted may continue in the circulation, acting merely as foreign bodies, for some time after their formation. The pathogenic effects appear, as in other hæmatozoa, to be caused by toxic substances set free during the stage of multiplication.

In addition to the forms described, others are sometimes observed. These consist of slight variations from the usual motionless type, the trophozoite. Large oval or even spherical parasites are seen, with banded nuclei, sometimes free, more often in leucocytes, and rarely showing signs of a cyst wall (pl. II). Such parasites are common in liver smears, but rare in the circulating blood. No evidence of sexual differentiation of the trophozoites has been observed, unless the forms just described be regarded as sexually different from the usual type. It seems more probable that these large parasites are very young schizonts, which occasionally escape from the liver after partial development, into the blood stream.

NONSEXUAL MULTIPLICATION OF *H. PERNICIOSUM* IN THE RAT.

It has already been mentioned that the trophozoites found in the large lymphocytes are practically uniform in size and appearance. No form is seen which may be construed as young or partly developed. The explanation of this lies in the fact that multiplication and development into mature parasites takes place in the cells of a single organ, the liver. At an early stage of infection, when parasites are numerous in the blood, a section of the liver shows many parasites in various stages of schizogony. Similar forms have been found in no other organ, although carefully sought for. The trophozoites are especially abundant in the spleen, kidney, and brain, but there is no evidence of multiplication in these organs.

In the capillaries of the liver are many large lymphocytes containing encysted parasites similar to those encountered in the peripheral circulation. In addition, there are parasites whose contour blends with the protoplasm of the leucocyte and appears to be unprovided with a cyst (pl. II). Free parasites, some of which are large and oval in shape (pl. II), are also abundant.

The multiplication of the parasite takes place in a liver cell. The earliest stage is a small spherical form 10 micra in diameter, with a large vesicular nucleus composed of chromatin granules in a fine mesh work, and a large karyosome. It is embedded in the protoplasm of the liver cell, the nucleus of the latter being pushed to one side. The cavity formed in the liver cell is fully occupied by the parasite. As development proceeds the parasite increases in size and the nucleus divides into two. This stage is known as schizogony and the parasite becomes a schizont. Many small, round granules are found in the latter when viewed in the fresh condition. The two nuclei divide and subdivide until from 12 to 20 daughter nuclei are formed. The schizont increases in size and the liver cell becomes a mere shell, the nucleus of which, after undergoing the changes characteristic of degeneration, finally disappears. A delicate cyst wall is formed around the developing schizont, which, at maturity, is ovoid and measures, on the average, 25 by 30 micra. Some, however, are larger and may measure 28 by 35 micra.

The nuclei of the schizont are arranged at the two extremities, as shown in longitudinal sections (pl. III). In fresh cysts studied at this stage the central mass of protoplasm is granular, with a clear area at the ends. At a later stage short rods of clear protoplasm, each containing a nucleus, are seen at the poles. These rods increase in length, the proximal extremities growing toward one another; the central granular mass diminishes in size. Eventually the clear rod-shaped bodies have become fully grown merozoites and occupy the entire cyst except for a small granular "rest" body. When the merozoites are fully grown, the cyst containing them becomes soft and gelatinous; later it ruptures and the merozoites escape, becoming free moving vermicules. The number of merozoites is from 12 to 20; the average 16.

In scrapings made from the cut surface of a liver in which the schizonts and cysts are numerous, their structure and the ease with which they are ruptured is readily determined. In sections from tissue, fixed and embedded in the usual method, the contents of the cyst shrink to a greater degree than the liver tissue and artificial spaces are formed around the schizonts (pl. III, fig. 1).

In heavily infected livers the number of cysts is very large. Their presence is associated with extensive fatty changes in the liver parenchyma. When a small piece of fresh liver tissue is spread out thinly between slide and cover slip and examined with the microscope on a warm stage, the contents of the ripe cysts are seen to be in motion. The merozoites move slowly about, gliding over one another, like a mass of worms. Often such a cyst is seen to rupture and the merozoites escape. In this event they move about as free vermicules, bending and twisting and gliding about exactly as will be described later in the case of vermicules formed from sporozoites, from which they can not be distinguished. Some of these vermicules enter the liver capillaries and later the circulating blood; others enter fresh liver cells and repeat the process of multiplication.

In stained smears made from the liver the various stages in the transformation of the free vermicule into the encysted trophozoite are readily traced (pl. II). In the former the nucleus is composed of large chromatin granules and the parasite is slender and elongated. It then becomes contracted in length and oval in shape; still later the nucleus is disposed in the characteristic parallel skein arrangement, and finally a cyst develops, the parasite being engulfed by a large mononuclear lymphocyte. No evidence of sexual dimorphism of the schizonts has been noted. The merozoites, when released from the cysts, are fully developed and do not subsequently increase in size in the blood. The large mononuclear leucocytes are found in in unusual numbers in the liver, in the sinuses of the lymphatic glands, the thymus, spleen, and bone marrow.

There seems to be little doubt that the vermicules, which are taken in by leucocytes in the liver and elsewhere and become encapsulated trophozoites, undergo no further development in the rat. Those vermicules which escape the phagocytic action of these cells and enter other liver cells repeat the process of schizogony. The majority perhaps are carried into the peripheral circulation in the leucocytes.

The liver cysts and merozoites are almost exactly duplicated in size and appearance by the sporocysts and sporozoites in the body of the intermediate host, to be described later. Encysted trophozoites are especially abundant in the spleen, lungs, kidneys, and brain, in the blood vessels, and lymph spaces.

CLINICAL SYMPTOMS IN WHITE RATS.

When the infection is slight, the rat often presents no evidence of disease. For a month or so the blood may show only a few parasites encysted in the leucocytes. This type of the disease is more common in old rats.

Frequently the disease begins gradually, with a few parasites in the blood. With the gradual increase in the amount of infection the animal becomes more and more anemic. It may continue in this state for two or three weeks and then gradually recover, or death may occur. Other animals, especially those experimentally infected, may die three or four days after the appearance of parasites in the blood; anemia rapidly becomes marked and the number of leucocytes and parasites is very great. Occasionally many free vermicules are observed shortly before death.

In rats with heavy infection there is marked apathy, little food is taken, and the animal sleeps constantly, with the head tucked under the thorax.

The anemia is most striking. The ears and skin, as well as the mucous membranes, become pearly white. When blood is drawn from the tail it is thin and watery, and it is very difficult to make a smooth smear. When death occurs after a long period there is great emaciation. The temperature may rise as high as 38.6° C.

Shortly before death there is often diarrhea, with an occasional admixture of blood, and the temperature is subnormal. In one animal hemoglobinuria was observed.

Lethality.—Of the 17 rats originally found infected, 4 died. However, 4 which showed severe infection were chloroformed to provide material for inoculation and fresh tissue for sectioning. Of 16 rats infected experimentally, 8 died—a lethality of 50 per cent. Young rats appear to be most susceptible and the majority of the fatal infections were in animals not fully grown. In half-grown rats the disease is almost invariably fatal in a few days after the onset of symptoms.

PATHOLOGICAL ANATOMY.

The gross lesions are most marked in animals which succumb to infection. In chronic infections the animals are much emaciated. The muscles and mucous membranes are extremely pale. The heart's blood is thin and watery. The lungs frequently show minute hemorrhages upon the surface. The spleen is greatly enlarged and dark in color; it may be five or six times the normal size, and frequently reaches from the diaphragm diagonally across to the right side of the brim of the pelvis. The small intestine occasionally contains thin fluid blood. The mesenteric glands are enlarged, sometimes hemorrhagic. The liver is distinctly enlarged, dull yellow in color, sometimes mottled, and the thin borders are rounded. Smears and frozen sections show extensive fatty changes. The kidneys show moderate parenchymatous degeneration. The urine rarely contains hemoglobin; more often bile pigment. The bone marrow is red.

THE ARTHROPODA AS AGENTS IN THE TRANSMISSION OF DISEASE.

The sexual cycle of *Hepatozoon perniciosum* takes place in a parasitic mite (an acarien), *Lelaps echidninus* Berlese. Mr. Nathan Banks, of the United States Department of Agriculture, has kindly made the determination. From a careful search of the literature it appears that this is the first recorded instance in which a member of the family *Gamasidæ* has acted as the intermediate host for a protozoan parasite.

So far as accurate knowledge extends, when a parasitic protozoon requires two hosts for the completion of its life cycle one of these hosts will be found to belong to the phylum Arthropoda. An apparent exception exists in *Hæmogregarina stepanovi* in the turtle (*Emys orbicularis*), the invertebrate host of which is a leech (*Placobdella catenigera*).[a]

Of the five subdivisions or classes into which the phylum is divided, two—the Insecta and the Arachnida—include the species which act as intermediate hosts.

Of the insects which serve as parasitic hosts and agents of transmission for pathogenic protozoa the mosquito is the best known, and the malarial parasite the only form which has been accurately worked out in all stages.

The life cycle of malaria in birds (*Proteosoma grassii*) is almost as well worked out as malaria in man. Quite recently Christophers has described the complete sexual cycle of *Piroplasma canis* in the dog tick, *R. sanguineus*.

Of insects, a number, including biting flies, lice, bedbugs, and fleas, are known to be concerned in the transmission of blood parasites. Some of these have been proved to be the agents of transmission, but whether as true intermediate hosts or merely carriers is as yet undetermined, in many instances, the sexual cycle being unknown.

In the class Arachnida the ticks have constituted, up to the present time, the only group capable of transmitting protozoan disease. The number of known tick-borne diseases is increasing and they are of great importance. In an epoch-marking work upon Texas cattle fever Smith and Kilborne,[b] in 1893, were the first to show the part played by the Arthropoda (in this instance ticks) in conveying disease. Various forms of piroplasmosis, relapsing fever, Rocky Mountain spotted fever, and *Leucocytozoon canis* have been shown to be transmissible by ticks.

Until quite recently it was believed that ticks transmit infection only through their progeny; i. e., only ticks derived from the eggs of infected mothers have power to inoculate susceptible animals. Recently it has been shown that infection with certain kinds of piroplasma is taken during the nymphal stage, but is transmitted only by the adult tick.

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[a] See p. 9, ref. *b*.

[b] Smith, Th., & Kilborne: Texas fever. Bull. No. 1, Bur. Animal Indus., U. S. Dept. Agric., Wash., 1893.

PARASITIC MITES.

Since but little is generally known of the nature and structure of mites, a brief account of their habits and a detailed description of the anatomy of the mite (*Lelaps echidninus*) which is concerned in the transmission of *H. perniciosum* is given below.

The Acarina, an important group of the Arachnida, comprise the mites and ticks, the former being greatly in the majority as regards number of species. The term "mites" is often applied to the entire group of Acarina. In addition to their arachnid characteristics (the absence of antennæ and the possession of four pairs of legs) the Acarina are distinguished by the absence of a division into thorax and abdomen, and by the arrangement and structure of the mouth parts, which are adapted for puncturing and sucking. The larva resembles the parent except in the possession of three pairs of legs and the absence of sexual organs. After casting the skin two or more times (ecdysis) the adult stage, with four pairs of legs and fully formed sexual organs, is attained.

Canestrini's[a] classification, from a scheme devised by Kraemer, divides the Acarina into six subheads:

Astigmata (Sarcoptic and vermiform mites).
Hydracarina (Water mites).
Prostigmata (Harvest mites).
Cryptostigmata (Orbatid mites).
Metastigmata (Ticks).
Mesostigmata (Gamasid mites).

A number of classifications varying in slight degree have been based upon this original. The following classification of the Acarina is from Neumann:[b]

Nonvermiform Acarina	Legs inserted directly into the integument without epimeræ....	Legs with five articles			*Oribatidæ.*
		Legs with six articles	With tracheæ. Cheliceræ....	Provided with hooklets	*Ixodidæ.*
				Didactylous or styliform	*Gamasidæ.*
			With no tracheæ (marine forms)		*Halacaridæ.*
	Legs articulated on distinct epimeræ	Natatorial legs (aquatic)			*Hydrachnidæ.*
		Ambulatory legs....	Cheliceræ styliform; palps free, antenniform		*Bdellidæ.*
			Cheliceræ styliform or in claws; palps free, ravishers		*Trombididæ.*
			Cheliceræ didactylous; palps cylindrical or conical and partially adherent to the tips		*Sarcoptidæ.*
Vermiform Acarina	Legs with five articles				*Phytoptidæ.*
	Legs with three articles				*Demodecidæ.*

Of these ten families only five are parasitic—the *Ixodidæ* (s. l.), *Gamasidæ, Trombididæ, Sarcoptidæ,* and *Demodecidæ.* Many of these are commensals, obtaining nutriment from the organic detritus, epithelial scales, hair, feathers, etc., of the host. The last two families are chiefly of interest to the veterinarian as producing various forms of mange. Only two families are blood-sucking, the *Ixodidæ* (s. l.) and the *Gamasidæ*. The rôle of the former (ticks) in the transmission of disease has already been mentioned. A few species of the *Trombididæ* are known to attack man; a larval form (*Leptus autumnalis, L. americanus*) is the well-known "red bug" or "chigger," also known as the "harvest mite" in England and the "bête-rouge" in South American countries. In Japan the Tsutsugamushi disease or Kedani disease, frequently fatal, is due to the bite of the "akamushi" or "red mite." This disease has recently attracted considerable attention; the etiology is unknown.

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[a] Canestrini, S.: Prospetto dell' acarofauna Italiana., Padova, 1885–1897.

[b] Neumann, L. G.: Traite des maladies parasitaires non microbiennes des animaux domestiques. Transl. by Fleming. Ed. by J. McQueen. 2d ed. New York, 1906.

Among the *Gamasidæ* the "chicken mite" or "red spider louse" frequently bites those who visit houses in which fowls are kept. The *Gamasidæ* form a larger and more widely distributed family than the *Ixodidæ* (s. l.), but by reason of their small size and apparent harmlessness they have been but little considered. A number of species live on decomposing vegetable and animal matter, others act as commensals upon beetles, reptiles, birds, and small mammals. *Gamasidæ*, which are true bloodsuckers, are found upon lizards and snakes (*Ophionyssus natricis* Megnin); upon vipers and water adders they attack the cornea, rendering it opaque, the reptiles becoming blind in consequence and dying of starvation. *Dermanyssus gallinæ* is a great pest to poultry and horses and even attacks man, withdrawing blood through the punctures made by its pointed mandibles. A number of species infest sparrows, canary birds, and parrots. A great number of small mammals are affected, including rats, field mice, moles, rabbits, etc. Upon bats a very large species (*Pteroptus vespertilionis*), popularly believed to be a bedbug, is found. The number of species of *Gamasidæ* is legion, and doubtless when more attention is paid to this family many others will be discovered.

THE GAMASIDÆ.

The following brief description of the structure and habits of the *Gamasidæ* is taken in part from Banks.[a]

In general the *Gamasidæ* are broad and flat, with a chitinous integument. Eyes are absent, but numerous sensory "hairs" on various parts of the body make up for this deficiency. Many forms are rapid in movement when disturbed. The mouth parts in many species can be withdrawn into the body. The head or capitellum can also be considerably retracted. The mandibles, two in number, are usually chelate, with toothed fingers. In some mites they are styliform. In the male the mandibles usually present specific differences.

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[a] Banks, N.: A treatise on the acarina or mites. Proc. U. S. Nat. Mus., vol. 28, pp. 1–114, 1904.

Beneath the mandibles is a projection called the hypostome. Sometimes an epistome is present above the mandibles. Projecting from either side of the head or capitellum are the palpi, which are provided with sensory organs.

The inferior surface of the *Gamasidæ* is provided with a varying number of chitinous shields or plastrons, upon which are located the genital apertures. The legs are six jointed, being divided into coxa, trochanter, femur, patella, tibia, and the terminal tarsus, provided with hooks or sucking disks. The female genital opening is usually between the sternal and genital plate. In the male it is in the sternal plate.

The act of coition is very remarkable and has been described in detail by Michael.[a] Briefly, it consists in the deposition by the male of a mass of spermatozoa (often contained in a delicate cyst) into the spermatheca, a sac connected with the uterus in the female. The spermatozoa upon liberation fertilize, from time to time, the ova as they become ripened. *Gamasidæ* lay comparatively large eggs, from which, with a few notable exceptions, six-legged larvæ are hatched. In the genus *Lelaps* the eggs, when deposited, are fully one-eighth the size of the female. One egg only is deposited at a time. It contains a fully formed embryo with six legs and fully developed nervous and digestive system. Very soon after expulsion from the female the delicate membrane or shell which incloses the embryo is ruptured and a six-legged larva appears. After several moultings of the skin the adult is formed. The immature mites are termed nymphs and, according to the number of moults gone through, protonymphs, deutonymphs, tritonymphs, etc. In the nymphal stage many of the *Gamasidæ* are migratory. Often they attach themselves to beetles, mosquitoes, and other insects, it is believed for the purpose of distribution.

LIFE HISTORY OF *LELAPS ECHIDNINUS*.

The species of *Lelaps* (*L. echidninus* Berlese) found upon white rats is a true parasite and sucks the blood of the animal. Its habits differ greatly from those of ticks in that its depredations are constantly repeated and only a small amount of blood is taken at each feeding. The mite is but slightly distensible, hence its capacity is limited. The following biological observations have been made in connection with the studies on *Hepatozoon perniciosum:*

When rats are kept in a cage of some size, in which there is an abundance of straw, the mites appear to feed almost exclusively at night and during the day they leave the host and live in the damp straw where the eggs are deposited. Hence when a number of rats are in the same cage the mites may visit a different animal each night. It is an easy matter to cause the mites to get upon the rats in the daytime by stirring the straw about in the cage and making the rats move about. In small cages, such as the glass jars used in the laboratory for holding single animals, the mites behave differently, especially if sand or sawdust is placed in the bottom. The rat moving about frequently in the narrow cage keeps the mites constantly disturbed, and they remain upon their host all the time and may be found in large numbers upon the back of the animal near the root of the tail. The female mite after feeding leaves the rat to deposit her eggs, which are laid one at a time, in damp straw. Each egg contains a well developed embryo. After hatching, the six-legged larva in a few hours moults and becomes a nymph with eight legs. The nymphs, though active, are seldom found upon rats and do not appear to suck blood. The nymphs after two moultings become adult. The young adult females (at first colorless) seek the host, and draw blood from small wounds made by the maxillæ or mandibles. When fully grown they are larger and brown in color. The males do not suck blood, but feed upon animal or vegetable detritus. They are few in number upon rats. The average length of life of the female mite is about six weeks. During this period a number of feedings of rat's blood are necessary to provide material for the development of the embryos.

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[a] Michael, A. D.: British Tyroglyphidæ. Ray. Soc. I, 1901, 291 pp.; II, 1903, 183 pp.

EXTERNAL ANATOMY OF *LELAPS ECHIDNINUS*.

TECHNIQUE.—In view of the small size of the mite nearly all the details of structure of the exo-skeleton may be determined by examination with low powers of the microscope after a few minutes immersion of the live mite in alcohol.

For brief study the specimens may be mounted in water on a slide with a cover glass. For examination with higher magnifying powers mites must be macerated in caustic potash to render them more transparent. The details of the mouth parts may often be studied in live specimens to advantage, the capitellum being then more markedly protruded.

GENERAL BODY CONSTRUCTION.—The head region or *capitellum* (pl. IV) is distinctly separated from the thorax and contains the mouth parts and certain appendages. The fused thorax and abdomen are covered dorsally by the *scutum,* a single chitinous plate. On the inferior or ventral surface are three chitinous plates—*sternal, genital,* and *anal.* The sternal plate serves as a point of attachment for the legs. The latter are eight in number (four pairs) and are composed of six articles, terminating in two hooklets and a sucking disk.

The capitellum is provided with paired sharply pointed *maxillæ,* and it incases for a portion of their length the paired *mandibles,* which are provided with didactylous pinchers in the female. In the male they are modified to form "clasping" organs. The *maxillary palps* are attached, one on either side, to the lateral aspect of the capitellum. They serve as sense organs, eyes being absent, and are composed of five articles. Two *stigmata* are present, with a prolongation forward of a tubular *peritreme.* The *anus* is located in an anal plate, the anterior margin of which is convex and the posterior prolonged into a large spine. In the female the *sexual aperture* is located in the mid-line of the body in the genital plate, near the posterior border of the sternal plate. In the male the *genital pore* is in the center of the sternal plate. The body of the adult female measures from 1.1 to 0.9 mm. in length by 0.7 to 0.6 mm. in breadth. The male is two-thirds the size of the female.

INTERNAL ANATOMY OF *LELAPS ECHIDNINUS.*

TECHNIQUE.—The difficulty of dissecting so small an object as this mite, especially as it is covered with hard chitinous plates, is very considerable. With the aid of fine needles, teased preparations can be made, a small per cent of which will be fairly successful. In the main, serial paraffin sections must be relied upon. The fixatives used were 5 per cent formalin, 80 per cent alcohol containing 5 per cent of formalin, and alcohol in various strengths. In addition, a fixative composed of 3 parts chloroform, 2 parts glacial acetic acid, 1 part saturated solution of bichloride of mercury, and 6 parts absolute alcohol was employed. In some instances soaking in a solution of 2 per cent celloidin preceded the embedding in paraffin.

Some satisfactory sections parallel to the ventral surface were made without softening the chitin, since none is encountered by the knife. For other sections in which the chitinous plates are to be cut, the mites, previously exposed to the action of fixatives, must be treated with a softening solution. For this purpose Labaraques solution in varying dilutions was employed. The length of time of its action depends upon the strength of the reagent and can only be determined by trial. It was found preferable to merely soften the chitin rather than to completely remove it. In the latter event, air bubbles form in the body cavity, which greatly interfere with subsequent dehydration and embedding.

The sections, affixed to slides with Mayer's albumin fixative, were stained with Weigert's iron hematoxylin.

ANATOMY OF THE HEAD REGION.—The capitellum is prolonged anteriorly to form the mouth parts. The dorsal anterior border forms a serrated projection, the *epistome.* The mandibles, which are double jointed and provided with muscles, terminate in pinchers with serrated jaws in the female. In the capitellum they lie in chitinous sheaths and are prolonged posteriorly, to be attached by tendinous expansions to the under surface of the scutum. Muscles attached to the same point serve to retract or protrude the mandibles.

The ventral aspect of the capitellum terminates in a bifid *hypostome* or maxillæ (pl. VI). The maxillæ are situated, one on either side of the mouth opening and form part of its lateral boundary. They are acutely pointed and serve as piercing organs. Between and dorsad of the maxillæ is a single elongated organ, the *lingula* or tongue (pl. VI), which presents a median groove. Its surface is covered with minute papillæ. The outline of the mouth opening is somewhat triangular, the borders being formed by fringed membranous folds.

THE PHARYNX.—The *pharynx* is situated just posterior to the mouth, in which it terminates. It lies almost entirely in the capitellum, forming a canal in the lower portion. It is T shaped (pl. VII, fig. 2) on cross section and its walls are connected by a series of transverse muscular bands with the chitinous covering of the capitellum. These muscles serve to pull apart the walls of the pharynx and produce suction. Interspersed with the transverse muscles are a series of circular muscular bundles (pl. VII, fig. 1) capable of constricting the pharynx.

THE ESOPHAGUS.—Posteriorly the pharynx terminates in a slender tube, the *esophagus* (pl. VII, fig. 1). This in turn communicates with the *ventriculus* or stomach. Surrounding the esophagus is a large *ganglion* or brain.

The ventriculus is a thin-walled sac consisting of a central and two lateral portions (pl. VIII, fig. 2). The former communicates anteriorly and inferiorly with the esophagus. The diverticula are prolonged anteriorly and posteriorly and communicate with the lateral aspect of the ventriculus. The wide central portion (pl. VII, fig. 1) extends from the brain to the middle third of the thorax, resting upon the inner skeletal plate. Regarding each lateral diverticulum as two projections (anterior and posterior) from the ventriculus, there are in addition two others, viz, the median anterior projection to join the esophagus and a posterior projection terminating in the *colon*, which, in turn, empties into the *excretory bladder*. When the uterus contains a large embryo the ventriculus is somewhat distorted and displaced.

The ventriculus and its lateral prolongations are lined with a single irregular layer of large epithelial cells with faintly staining reticular and vacuolated protoplasm and inconspicuous nuclei. The cells rest upon a delicate basement membrane. Encircling the digestive tract are a series of minute parallel muscular fibers, each one isolated and with a single nucleus. These fibers serve to constrict the ventriculus and its diverticula, and in young live mites with transparent scutum the waves of contraction, beginning anteriorly and sweeping backward, are easily discerned. Adherent to the lining layer in some places, but often free in the lumen of the ventriculus, are large spherical cells, sometimes called "liver" cells. They possess very small faintly staining nuclei, excentrically located, and are phagocytic in nature, often containing cell inclusions of various kinds, vacuoles, and yellow pigment. According to Berlese[a] they secrete a digestive ferment and play an important part in the digestive process. That portion of the body not taken up by the digestive and other organs is occupied by muscles which move the legs and mandibles and by fat cells.

The *colon*, a short, thin-walled tube, terminates in an *excretory bladder*, which communicates with the anus. Opening into the lateral aspect of the excretory bladder are paired tubes—the *malphigian* vessels—which run forward parallel beneath the ventriculus almost to the capitellum, to end blindly. They serve as excretory organs, and often contain small dumb-bell shaped crystals of mineral salt.

The *salivary* glands are paired structures in the body cavity, situated beneath the anterior extremity of the scutum (pl. VII, fig. 1). Each gland is pyramidal shaped and composed of several acini, which are formed of large cells of coarse granular structure, with small, indistinctly defined, deeply staining nuclei. The apex, which is below, terminates in a minute duct which empties into the posterior part of the pharynx. Each acinus contains a central cavity, which varies in size according to the activity of the gland. Small accessory glands are sometimes observed.

SEXUAL ORGANS.—The *ovary* is an irregularly shaped, oval body situated in the posterior portion of the body. It is composed of a reticular framework, in which are many young *ova* and small cells which form the stroma. Projecting from its anterior and lateral aspects are ova in various stages of development. Three or four ova in advanced stages of development, one of which is fully ripened, may usually be observed.

In adult females the *uterus*, a thin-walled sac lying in front of the ovary, usually contains an embryo which may show complete development. The ripe ovum is fertilized in the uterus, which communicates by a narrow vagina with the genital aperture. The latter is covered by finger-like sensory organs (pl. IV, fig. 2). Attached to the top of the ovary are peculiar tubular structures—the *lyrate organs*—to which are connected a pear-shaped sac (*sacculus fœmineus*) containing spermatozoa. The lyrate organs pass on either side of the uterus to terminate upon the inner aspect of the acetabula of the coxæ of the third pair of legs.

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[a] Berlese, A.: Ricerche sugli organi e sulla funzione della digestione negli acari. Riv. di Pat. Veg., Terenzi; vol. 5 (5–8), 1896, pp. 129–195.

Since only the female mite is directly concerned in the transmission of *Hepatozoon perniciosum*, the anatomy of the male sexual organs will be only briefly considered. The *testis* is a single oval body situated posteriorly between the ventriculus and the excretory bladder. The paired *vasa deferentia* pass downward from each lateral aspect, then forward near the ventral surface, to terminate in the single genital opening in the center of the sternal plate. The *spermatoza* are large, round or elongated, granular structures, and are usually found in the sacculus fœmineus in the female. Here they are stored until the ova, becoming ripened one at a time, are fertilized.

TRACHEAL SYSTEM.—The stigmata are placed on the lateral aspect of the body, between the third and the fourth pair of legs. A tubular prolongation forward, the *peritreme* or *stigmal canal* (pl. IV, fig. 2) divides into the main tracheal tubes, which ramify throughout the body, some terminating in the legs. The tracheal system is absent in the larvæ.

The following articles may be consulted for a more detailed account of the structure of the *Gamasidæ:*

Winkler, W.: Anatomie der Gamasiden. Arb. a. d. Zoolog. Inst., Wien, vol. 7, part 3, 1888, pp. 1–38.

Berlese, A.: Recherche sugli organi e sulla funzione della digestione negli acari. Riv. di Pat. Veg., Firenzi, vol. 5 (5–8), 1896, pp. 129–195.

Michael, A. D.: On the variations in the internal anatomy of the *Gamasidæ*, especially in that of the genital organs, and on their mode of coition. Trans. Linnean Soc. of Lond., 1892.

Mégnin: Memoire sur l'organization et la distribution zoologique des acariens de la famille des *Gamasides*. Journ. d. l'Anat. et de la Physiol., 1876.

Kramer, P.: Ueber Gamasiden. Arch. f. Naturg., vol. 1, 1882.

Michael, A. D.: British Tyroglyphidæ. Ray. Soc., I, 1901, 291 pp.; II, 1903, 183 pp.

SEXUAL CYCLE OF *HEPATOZOON PERNICIOSUM* IN THE MITE.

Mites removed from rats (in whose blood parasites were abundant) were found, in the majority of cases, to be infected with *H. perniciosum*. Such mites, having fed upon the rat's blood from time to time, usually showed the parasites in several stages of development. Cross sections of mites pictured in pls. IX and X show this condition. Frequently, when many fully developed parasites were present, young forms also were observed in the stomach, indicating a fresh infection with each feeding of blood. Mites which were heavily infected with fully developed parasites could often be distinguished by the naked eye from normal mites. In the former the lateral and caudal portion of the body is distended and pearly white, whereas in the latter this part is dull brown in color.

In order to determine the length of time required for the various stages of development of the parasite, as well as to study them in detail, a number of mites were artificially infected, or, more properly, the usual manner of infection was controlled. For this purpose it was necessary to have a supply of normal mites. Fortunately these could be readily obtained. The stable in which the infected rats had been kept contained a large room in which were a number of rat cages. In two corners large single isolated cages had been built. One of these contained infected rats; the other had been emptied of rats about two weeks. Upon examination both were found to contain damp straw and grain, upon which were myriads of mites. In the empty cage the mites were found to be free of infection. Six healthy rats, fresh from the dealer, were placed in this cage. In a very short time each rat was infested with a large number of mites.

To remove the mites, which run about with considerable rapidity upon the hair and skin, the rats are seized by the loose skin at the nape of the neck with the tip of a pair of long clamps. The tail of the animal is grasped with one hand and by traction the body is extended along the blades of the forceps. In this position it is easy to remove the mites with a fine-toothed comb, from which they are quickly shaken by a tap of the finger into a large glass jar. The inside of the rim of the jar should be previously smeared with a ring of vaseline, which the mites will not traverse. In this manner it is possible in a short time to collect several hundred.

The mites are kept in the jar several days to starve them and then placed upon infected rats. For this purpose four rats were selected in whose blood the parasites were very numerous. To transfer the mites to a rat it is only necessary to place the latter in the jar for a few minutes. In a short time all the mites will have crawled upon the rat and concealed themselves in the fur.

After six, twelve, eighteen, twenty-four, thirty-six, and forty-eight hours, mites were removed and examined in fresh preparations and stained smears, and a number preserved in fixing solutions. From these paraffin serial sections were made. After forty-eight hours a large number of mites were removed and placed upon healthy rats in order that the parasites then present in the mites might develop without admixtures of younger stages, which would have been the result of continued feeding upon the diseased rats. At intervals of twenty-four hours a number of these mites were removed and examined and sections made. Successive collections of mites were fed upon infected rats in order to obtain a large number of infected mites for later experiments.

In order to study the effect of the digestive juices of the mite upon encysted parasites in the rat's blood, the following experiment was performed a number of times. The various stages of the process could be conveniently followed with the microscope for an hour or more.

Blood from a rat containing numerous parasites encysted in lymphocytes was mixed with the expressed body juices of mites, placed upon a slide, and examined with the microscope. In the course of ten to thirty minutes the protoplasm of the lymphocyte was partly dissolved and the parasites were set free.

The encysted parasites, after becoming free from the leucocytes, are slender, elongated bodies, rounded at the extremities. They show no movement or change of form. After thirty minutes or longer the delicate cyst wall surrounding them is ruptured and the parasite becomes an actively motile vermicule. The remains of the cyst, as a transparent shadow, often continues to be attached to the vermicule for some time.

The same changes may be observed when a mite that has just fed upon blood is crushed and the expressed fluid examined.

When mites starved for forty-eight hours or longer are examined six hours after being placed upon heavily infected rats, numerous free vermicules are found in the stomach and its diverticula. When these mites are crushed under a cover slip in a drop of 0.3 per cent salt solution, the vermicules and a considerable number of polymorphonuclear leucocytes are observed. Other kinds of leucocytes appear to be quickly destroyed. Not infrequently the remains of the delicate cyst which inclosed the parasite is seen attached to some portion of the vermicule. In form and appearance the vermicules do not differ from those developed from the sporozoites. The movements, however, although fairly active at first, become quite sluggish, and in mites examined eighteen hours after feeding, motile forms are seldom seen.

The red blood cells disappear very quickly after the ingestion of the blood, but hematin crystals, polymorphonuclear leucocytes, and free parasites are abundant. The polymorphonuclears are especially resistant and appear to remain unchanged after a number of hours in the digestive tract of the acariens. The parasites become detached from the lymphocytes when these are digested. In mites examined twenty-four hours after feeding, the digestive fluid contains single and paired vermicules in which movement has ceased.

The form and arrangement of the paired vermicules is peculiar and characteristic. In the earliest stages they are somewhat spindle shaped and two extremities are in apposition (pl. XIII, fig. 1). This constitutes the earliest phase of conjugation or sexual union. A little later the vermicules are placed side by side and parallel, the extremities even. In appearance they are exactly similar, and hence without distinguishing sexual characters. In slightly later stages the adjoining sides are quite straight and parallel and separated by a slight interval (pl. XIII, fig. 2), although apparently attached in some manner, since their relative position is unchanged by movement of the fluid containing them. Next the two become more rounded and increase slightly in size. The nuclei are large and vesicular and the dividing line still persists (pl. XIII, fig. 3). Later one vermicule grows longer and larger and begins to partly encircle the smaller, which becomes more rounded (figs. 4–7). They may now be properly regarded as gametes. By analogy the smaller and more finely granular is the microgamete, the larger and more coarsely granular the macrogamete. Eventually the line of demarcation between them disappears. The zygote thus formed by fusion of the male and female elements, is slightly elongated, tapered at one extremity, with granular protoplasm and two large vesicular nuclei. The latter approach one another, touch, and become blended. After the union of two sexual elements in the manner described the zygote becomes more elongated (figs. 11–13) and exhibits very slow movements of flexion and extension and something resembling ameboid motion. The term ookinet is applied to such a zygote.

The ookinet continues to increase in size and becomes more and more markedly granular, especially around the nucleus, which contains a small karyosome. When first formed the ookinet measures about 10 by 25 micra. It does not appear to detach any of its substance nor does it contain pigment. From this point development is rapid. The ookinet becomes an elongated or oval body with granular protoplasm and a vesicular nucleus containing a moderate sized karyosome (fig. 13). The nuclear network is quite distinct in both fresh and stained preparations. In mites forty-eight hours after infection the ookinet has reached full size, which averages about 25 by 50 micra.

In sections of mites made at this period the ookinets are fairly numerous in the lumen of the gut and appear in many instances more or less curled up (pl. XII). A similar form is noted in fresh preparations, as well as short spindle and ovoid shapes. They often appear somewhat flattened and occasionally very long and slender. In mites, three and four days after feeding on infected rats, many of the ookinets have disappeared, having doubtless been passed out of the intestinal tract with the feces. A few, however, are adherent to the epithelium of the gut or embedded in the gut wall between the epithelial cells. The protoplasm is distinctly granular and stains much more deeply with iron hematoxylin than do the cells lining the digestive tract. The karyosome is prominent and deeply stained. Some parasites are spherical and lie directly upon the basement membrane, surrounded by epithelial cells. Others are seen outside the stomach wall in the body tissues of the mite. They are granular and deeply colored by basic stains.

The exact manner in which the basement membrane is penetrated by the parasite is unknown. Probably the contraction of the delicate muscular envelope of the alimentary sac causes the parasite to be forced through the spot, weakened by its encroachment upon the basement membrane. At any rate the ookinets are found free in the body cavity. As shown in the description of the anatomy of the mite the body cavities represent the numerous small spaces between the various organs, and between these and the chitinous body envelope. In any of these spaces, in the investing membrane of the salivary glands, or within the sheaths of the muscles inclosed in the body of the mite, the ookinets may be found.

After thus wandering about in the body tissues the ookinets become stationary and undergo a remarkable change. Coincident with an increase in size of the plasma, in which large round granules begin to appear, the karyosome becomes greatly enlarged, exceeding in size the original nucleus. The nuclear membrane is but slightly larger than this giant karyosome, which may measure 10 micra in diameter. When the parasite has reached a diameter (in the fresh specimen) of about 60 to 75 micra, it becomes oval or spherical and a delicate cyst wall makes its appearance. This stage is observed in mites four and five days after infection. It is evident that the large karyosome is the forerunner of active multiplication of the nucleus of the encysted parasite, which is now termed a sporont (Lühe) and the cyst an oocyst.

In sections of mites showing more advanced stages, the parasite often appears oval or flattened by reason of the restraining action of the various organs or tissues upon its growth. In fresh dissections of mites the sporonts are conspicuous by reason of their size, spherical form, and granular structure (pl. XIV). The cyst wall is often very much larger than the parasite it contains, the latter appearing to float freely within it. The sporonts at this stage measure from 100 to 150 micra in diameter. Upon reaching approximately this size the nucleus undergoes division into two. The division is repeated and the daughter nuclei migrate to a position near the surface of the protoplasm, which forms a perfect sphere (pl. XIV, figs. 1–2). Nuclear division continues until a large number of nuclei are formed, all located in a superficial layer of the protoplasmic mass. The oocyst reaches a maximum diameter of 200 to 250 micra, although usually somewhat smaller.

In mites infected six or seven days previously the surface of the sporont is covered with budlike projections (pl. XIV, figs. 3–4), each of which contains a nucleus. A little later the buds become more prominent and elongated and break off from the central mass. A large number (50 to 100 or more) of oval bodies are thus formed, each of which contain a nucleus. These bodies represent sporoblasts and measure about 10 by 15 micra.

When obtained in the fresh condition, by gently teasing apart mites in 0.3 per cent salt solution the sporonts are perfectly spherical or ovoidal (pl. XIV), with a delicate cyst larger than the sporont. In the large budding forms (pl. XIV) the central granular part becomes softened and gradually liquefied, the stored-up material being utilized in the growth of the sporoblasts. Very slight pressure is sufficient to rupture the sporont, causing the escape of the fluid contents and the collapse of the superficial portion with the attached sporoblasts. In sections this collapsed condition always exists, and the flattened sporonts on cross section show usually two rows of sporoblasts with the attached ends adjoining, suggesting a fern leaf in appearance (pl. XVII, fig. 5; and pl. XI).

The subsequent development of the sporoblasts is traced in various stages in pl. XVIII. The nucleus divides and subdivides, the daughter nuclei arranging themselves at the poles. A little later a cyst wall is formed around each sporoblast.

The nuclei become the nuclei of the sporozoites, the protoplasm of which is split off from the central mass as short rods (pls. XVI and XVIII), which increase in length and gradually envelop the diminishing central "rest-body," like fingers. With increasing growth of the sporozoites at the expense of the "rest-body," the latter is eventually reduced to a small, round, granular mass. The sporozoites fill the interior of the cyst, which measures on the average 25 by 30 micra. They are arranged in two general groups, occupying the two halves of the cyst, as shown in pl. XVI, fig. 12–14, the adjoining extremities of sporozoites interlocking. This arrangement does not always persist, however, since later they may be mixed up indiscriminately and retain merely a general direction corresponding to the long axis of the cyst. When the cysts are ruptured in salt solution, the individual sporozoites appear as cresent or club-shaped bodies (pl. XVI, fig. 1–2). The nucleus, spherical in form, is located nearer the smaller extremity. The thicker and more rounded end, as will be seen later, is the anterior. The protoplasm is highly refractile. The measurements are 14 micra in average length, by 5 micra in greatest breadth, tapering to 2 micra at the narrow posterior extremity.

The rest body persists as a round granular body about 6 micra in diameter. The fully developed cysts are found in mites from ten to twelve days after feeding. The number of sporozoites is from 12 to 20 in each cyst; 16 is the average; occasionally as many as 24 are encountered. The cysts which contain the larger number are elongated and often distinctly bent in the middle. In addition to these, small spherical cysts containing about 10 sporozoites are sometimes met with. The sporozoites in such cysts do not appear to differ from those in the other cysts mentioned.

When ruptured by pressure, the cysts split in the direction of greatest length, the edges curling up. In sections of mites (pls. IX, X, XIX) showing large oocysts containing sporoblasts or sporocysts the outlines of the large cysts are somewhat irregular and not clearly outlined. Also, the varying effect of the pressure of surrounding organs and the shrinkage and distortion in fixation and embedding cause the large cysts to assume a variety of forms other than spherical.

The invasion of the salivary glands by the cysts is particularly interesting. In some specimens the gland on one or both sides has in part or entirely disappeared and been replaced by the parasites.

The sporozoites have never been found free, however, in such sections nor in fresh preparations, but always inclosed in the sporocysts. The possibility of the transmission of the parasite through the salivary glands and their appendages will be considered later.

In the study of the various stages which have just been described sections from a great number of mites and fresh preparations from hundreds of both normal and infected mites have been utilized. The observations were repeated and verified many times.

The presence of the parasites in moderate numbers does not appear to be injurious to the mites. Mites have frequently been observed, with fresh blood in the stomach and ripe sporocysts in the body tissues. However, those found dead have often been very heavily infected.

EXPERIMENTAL INFECTION IN WHITE RATS.

Although Christophers has described what he regarded as the sexual cycle of *Leucocytozoon canis* in a tick, he states in a recent volume[a] that "the sexual cycle can not yet be said to be definitely known." Certainly the experimental transmission of hæmogregarines by arthropoda or by direct inoculation has not, so far as the literature records, been successfully accomplished.

In the present work it was recognized that the conditions for experimental attempts at transmission of *H. perniciosum* were extremely favorable, the principal host being, so to speak, a domesticated animal, and therefore readily handled. The intermediate host—the mite—could easily be obtained in large numbers and its action was susceptible of complete control. In fact, all the conditions of natural infection could readily be reproduced artificially.

As already stated, a large number of mites (several hundred) were infected by being placed upon heavily infected rats for forty-eight hours or longer and then transferred to healthy rats for ten to fourteen days. When examined at the expiration of this period about 50 per cent showed heavy infection with ripe cysts. When an infected mite was crushed between slide and cover slip in a drop of salt solution hundreds of sporocysts containing sporozoites were expressed (pl. xv). Only rarely were the sporocysts still contained in the large cyst (oocyst), the latter being usually ruptured in the process of crushing or dissecting.

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[a] Stephens, J. W. W., & Christophers, S. R.: The practical study of malaria. 3d ed. London, 1908, pp. 1–414.

With a view of obtaining a clew to the manner of infection of rats in nature, a number of experiments were undertaken to determine the effect of various substances upon the sporocysts and the sporozoites. The observations were made partly upon the warm stage of the microscope, but largely without its use; the weather at the time being extremely warm, additional heat was often found unnecessary.

1. Action of fresh rat's blood.—A drop of a healthy rat's blood was mixed on a slide with ripe sporocysts, covered with a cover glass, and ringed with vaseline. No change in the sporocysts took place nor was any movement of the sporozoites in them observed as long as the sporocysts remained intact. On the contrary, when the latter were ruptured by pressure on the cover glass and the sporozoites set free in the blood, marked changes took place. After about fifteen minutes the sporozoites assumed a more elongated form, the principal change of shape taking place in the broad extremity. This end became more slender and elongated (but still slightly wider than the other) and the tip somewhat pointed. The nucleus was visible as a spherical body about one-third distant from the smaller extremity. The movement earliest observed consisted of a gradual bending until the ends were almost touching, then a sudden spring-like resumption of the original shape (pl. xvi). Other sporozoites, after becoming bent, became slowly straightened, a peristaltic wave of contraction beginning anteriorly and a gradually increasing length of sporozoite becoming straightened. A longitudinal contraction of the entire parasite, which rendered it shorter and broader, was followed by slow contraction in a transverse direction, beginning anteriorly, this portion becoming longer and thinner as the wave passed backward and causing the sporozoite to be projected slowly forward. A bending simultaneous with the longitudinal contraction often occurred, and in the following transverse contraction straightening proceeded from before backward, the anterior slender portion often making a distinct angle with the clubbed posterior part.

With high magnifying powers of the microscope and indirect light from the condenser, minute striæ, some arranged in an oblique transverse and others in an oblique longitudinal direction, can be made out without much difficulty. These striæ are caused presumably by minute fibrils—the myocyte-fibrillæ commonly observed in gregarines, which are capable of contraction.

After the slide has been allowed to remain for ten or fifteen minutes in the incubator and is then examined on the warm stage, the sporozoites are found to have become actively moving bodies called vermicules. By a sort of snakelike or wormlike motion, rapid change of place occurred, the red blood corpuscles being vigorously moved about when the vermicule came in contact with them. The anterior extremity was always the one which had been the broadest and most rounded in the sporozoite. In the moving vermicule this extremity is more slender and pointed. The entry of vermicules into leucocytes was never observed. After two or three hours they became sluggish in movement and finally stationary.

2. Action of 0.5 per cent solution of pancreatic extract in normal salt solution.—When sporocysts are exposed to this solution the cysts are rapidly dissolved and the sporozoites set free. The latter very soon become contracted into rounded bodies and lose all motion.

3. The digestive juices of the mite.—Mites containing sporocysts were compressed between a slide and cover slip. The chitinous envelope was then removed and the cover slip replaced and pressed down with sufficient force to rupture the sporocysts and set free the sporozoites. The latter, after a short time, performed slow movements of flexion and extension, and, in addition, some sporozoites showed at times a slow gliding motion forward, with intervals of rest. When in motion a minute point of protoplasm could be seen projecting from the anterior end. The protoplasm showed no perceptible waves of contraction during this movement. Some of the sporozoites at times exhibited slight contractions. No movement took place inside the unruptured cysts.

4. Action of juice from the duodenum of the rat.—A healthy fasting rat was killed, the abdominal cavity opened, and a drop of the clear fluid contents of the lower part of the duodenum mixed on a slide with ripe sporocysts. Immediately upon examining with the microscope the sporozoites in the unruptured cysts were found to be in active motion, moving about with great vigor in the interior of the cyst and suggesting very strongly a mass of worms squirming over one another. After ten or fifteen minutes many cysts were seen to rupture and the sporozoites were set free as actively moving vermicules. They exhibited all the movements previously described as observed when sporozoites were mixed with rat's blood, except that they were intensified. This observation (repeated a number of times) upon the action of the intestinal juice upon the sporocysts and sporozoites pointed to the intestine as a possible point of entry of the infection, in rats, and led to subsequent experiments in this direction.

The first experimental attempts to produce the disease in rats were conducted with the intention of simulating the natural method of infection. Ten healthy white rats about three-fourths grown and just received from a dealer were selected. Each rat was placed in a separate glass battery jar with wire-gauze top. Careful examination failed to disclose any skin parasites except a few lice. Two blood smears were made each day on two successive days from each rat. In every rat the blood was found to be normal. Mites previously infected and showing in about 50 per cent ripe sporocysts when crushed, were placed upon eight of these rats. From 20 to 50 mites were placed upon each rat. Two rats were kept as controls, no mites being placed upon them. The rims of the jars containing these rats were smeared with vaseline and they were kept in a separate part of the laboratory away from the others. The results were as follows:

Rat No. 1.—On the nineteenth day first appearance of parasites in the blood as encysted forms in lymphocytes; leucocytes increased. Twentieth and twenty-first day: Encysted forms more numerous; animal shows marked anemia. Twenty-second day: Lymphocytes greatly increased; in addition to many encysted parasites, numerous free forms. In fresh preparations some of these are actively moving vermicules. Twenty-third day: Leucocytes 120,000, 82 per cent being large mononuclear leucocytes. In counting 200 leucocytes 381 parasites were counted. Animal died.

Post-mortem.—Muscles and skin extremely pale; blood watery; spleen greatly enlarged and dark, measuring 6 by 2 by .5 cm. Liver, yellow in color; punctate hemorrhages in lungs.

Rat No. 2.—Few encysted parasites on twenty-fifth day, gradually increasing in number until thirty-fifth day. Anemia slight. At that time 38,000 leucocytes, large mononuclears 56 per cent. In counting 200 leucocytes 32 parasites encountered. On fiftieth day infection appears to be about the same.

Rat No. 3.—Moderate number of free and encysted forms on twenty-second day. Infection increased until death on twenty-eighth day. Leucocytes, 132,000, large mononuclears 71 per cent. In counting 200 leucocytes 165 parasites observed; few free forms.

Post-mortem.—Lesions as in rat No. 1, except spleen slightly smaller and in addition there is enlargement of abdominal and mesenteric lymph glands.

Rat No. 4.—Infection first observed on twenty-eighth day; few encysted forms; gradually increasing anemia and number of encysted forms; death on thirty-eighth day. Leucocytes 110,000 on thirty-sixth day; large mononuclears 68 per cent. In counting 200 leucocytes 96 parasites observed; very few free; nucleated red cells abundant.

Post-mortem.—Lesions as in No. 1.

Rat No. 5.—Few encysted parasites on twenty-fifth day, but gradual increase up to fiftieth day. Leucocytes 28,000, 37 per cent large mononuclears. In counting 200 leucocytes 28 encysted forms observed; none free. Animal appears healthy; slight anemia.

Rat No. 6.—Few free and encysted parasites on nineteenth day. On twenty-fifth day greatly increased in number, both free and encysted; moderate anemia. On fiftieth day animal alive, but seems sick and is quite anemic. Leucocytes 98,000, 62 per cent large mononuclears; many nucleated reds. In counting 200 leucocytes 231 parasites observed: 157 free.

Rat No. 7.—Infection appeared twenty-seventh day; death thirty-first day; great anemia. On twenty-sixth day leucocytes 91,000. 59 per cent large mononuclears. In counting 200 leucocytes 101 parasites observed, mostly encysted.

Autopsy.—Spleen 6 by 1 by 0.4 cm. Other lesions as in No. 1, except punctate hemorrhages absent in lungs.

Rat No. 8.—Free vermicules only in small numbers on seventeenth day; encysted forms numerous on twenty-fifth day; death thirty-sixth day. Leucocytes 126,000, large mononuclears 78 per cent on thirty-fifth day. In counting 200 leucocytes 83 parasites, mostly encysted, were seen.

Autopsy.—Lesions as in No. 1, except slightly smaller size of spleen.

Rat No. 9—Control.—Examined every five days up to fiftieth day. No parasites found. Leucocytes 10,600, few nucleated reds.

Rat No. 10—Control.—No parasites up to fiftieth day. Leucocytes 5,800.

Control animals were repeatedly examined and found free of mites.

INFECTION IN WILD RATS.

In attempting to infect wild rats (*Mus rattus*) with *H. perniciosum* an obstacle was encountered, due to the difficulty of handling them and of keeping them healthy in captivity.

In the first experiment five large rats, averaging 250 grams in weight, were used. These rats had just been captured in a wire trap. They were placed in separate glass jars, to which infected mites had been added. Unfortunately all the rats died within a week from self-starvation and the effects of confinement.

For a second experiment six young wild rats, apparently of the same age and averaging about 100 grams in weight, were obtained. These were placed in a large wire cage, which in turn was placed in a large wooden cage containing infected mites.

Results: One rat died on the thirty-third day and another on the thirty-ninth day. Examination of the blood and organs showed severe infection with *H. perniciosum.* At the end of six weeks the remaining rats were all infected with the blood parasite, but in a mild degree. At the end of eight weeks the animals still show the parasite. The age of the rats at the beginning of the experiment is assumed to be about four months.

FEEDING EXPERIMENTS.

Ten rats were selected and placed in isolated glass jars with gauze tops. Each cage, as well as each rat, was sprinkled with "insect powder." All the rats were carefully examined and found free of vermin except a few lice. These disappeared within a few days after the "insect powder" was used.

Blood smears from each rat were examined on two successive days. The blood was normal and showed no parasites. Before the feedings with infected material all food was withdrawn for forty-eight hours. Mites containing ripe sporozoites were crushed singly or in small numbers at a time upon double-width glass slides. Immediately after crushing, examination with the low power of the microscope was frequently resorted to, in order to be certain of the abundance of ripe sporocysts. Pieces of moistened white bread were used to absorb the expressed juices of the crushed mites. After a thin piece of bread about 2 cm. square had received from 15 to 20 or more crushed mites and their juices it was gently compressed into a firm round mass and immediately fed to a rat. In this manner eight of the ten rats were fed. The other two were kept as controls. The eight rats were fed as described only once; afterwards ordinary food (bread and oats) was given.

Twenty-four hours after the infected bread was given, and every day thereafter, stained blood smears, and on occasion, fresh preparations, were examined. In the first twenty-four hour preparations, five rats showed a moderate number of actively moving free vermicules in the blood; they were also observed in stained smears and differed in no way from those previously described. The leucocytes were but little if at all increased in number; no encysted or other variety of parasites were observed. When next examined (third day) no parasites were observed in any of the smears examined. The later findings are given below:

Rat No. 1.—A small number of encysted parasites observed on the eighth day; on twelfth day moderate anemia; parasites greatly increased, also leucocytes; death on fourteenth day.

Post-mortem.—Enlarged spleen, fatty liver.

Rat No. 2.—Moderate numbers of encysted and a few free parasites observed on twelfth day; gradual increase in number up to fortieth day; animal shows moderate anemia and seems sick; encysted parasites numerous.

Rat No. 3.—Moderate number of encysted parasites on eighth day; tenth day marked anemia, many free and encysted parasites. Found dead on morning of twelfth day.

Post-mortem.—Hemorrhagic spots on surface of lungs; blood in small intestine; other lesions as in No. 1.

Rat No. 4.—Few encysted parasites on ninth day; moderate number on twelfth day. On fortieth day an occasional parasite observed; animal appears healthy.

Rat No. 5.—Small number of free and occasional encysted parasites on eighth day; tenth day encysted forms numerous; moderate anemia; death on twentieth day.

Post-mortem.—Spleen greatly enlarged; liver mottled yellow; many parasites in blood.

Rat No. 6.—Encysted parasites in small numbers on twelfth day; moderately numerous on twentieth day; animal sick. On fortieth day a few encysted forms still present; animal appears healthy.

Rat No. 7.—Few free and many encysted parasites on ninth day; more abundant on twelfth day; animal anemic, appears sick; leucocystes greatly increased. Increasing anemia and death on twentieth day.

Post-mortem.—Spleen four times normal size; liver enlarged and fatty; mesenteric lymph glands enlarged and hemorrhagic.

Rat No. 8.—Small number of encysted parasites on eleventh day; on fifteenth day considerably increased; anemia apparent. On fortieth day animal still alive, but quite sick and anemic.

Rats Nos. 9 and 10—Control rats.—Were examined in the same manner as the infected ones. No parasites found in the blood at any time. Animals healthy on the fortieth day.

On none of the rats were mites or other ecto-parasites found. The jars in which the rats were confined were placed at some distance in the laboratory from those containing infected mites and were further protected by a ring of vaseline.

In a later experiment, carried out in the same manner, five rats were used. Four were fed with ripe cysts on wet bread. The earliest appearance of encysted parasites in the blood was on the eighth day in two rats; in the other two on the tenth day. Vermicules alone were observed in three on the second day. One rat died on fifteenth; the other on nineteenth day.

In a still later experiment four young rats (2 months old) were fed as in the previous experiments. Encysted parasites were found in the blood on the tenth, twelfth, thirteenth, and fourteenth day, respectively. All four died within a week after the appearance of infection.

Remarks.—Parasites spoken of as "encysted" refer to those forms inclosed in cysts and usually contained in mononuclear lymphocytes.

In the first experiment the five rats in which free vermicules were observed twenty-four hours after feeding infected mites were those in which encysted parasites were first observed. It is probable that a more prolonged search for parasites in the blood of the remaining three rats would have resulted in the finding of a few at that time.

An attempt was made to infect four rats by feeding them livers from infected rats in which cysts (containing merozoites) were numerous. The result was negative. No free vermicules were detected in the blood during the first twenty-four hours, nor did any encysted parasites appear later.

Defibrinated heart's blood from an infected animal was injected into the peritoneum of two healthy rats, 1 c. c. to each rat. Blood smears previously made from the infected rats showed only encysted parasites. Result negative.

In a later experiment defibrinated blood in which there were a few free vermicules was employed. The results were also negative.

Small pieces of liver containing ripe cysts were gently rubbed up in a mortar with salt solution. The liquid, strained through sterilized gauze, was injected into the peritoneum of two rats. Result negative.

Several mites containing ripe sporocysts were gently crushed and rubbed in a mortar containing 0.3 per cent salt solution. The liquid, when strained and examined with the microscope, was found to contain many free sporozoites. A portion of the fluid was injected into the peritoneal cavity of two rats. Result negative.

ACTION OF CITRATE SOLUTIONS UPON ENCYSTED PARASITES.—Defibrinated blood from an infected rat was added in varying amounts to small test tubes containing 1 c. c. of 1 per cent solution of sodium citrate in normal salt solution. Two tubes were set aside at room temperature (about 30° C.); two tubes were placed in the incubator at 37° C. for twenty-four hours. The same method of procedure was also followed, using normal salt solution instead of soidum citrate solution. Results: In salt solution kept for three days at 30° C. a few sluggishly motile vermicules were found. In other solutions no change took place in the encysted parasites.

A series of sections were made from the middle and lower part of the small intestine of a fasting rat, fed twelve hours before, on a large number of crushed mites heavily infected with ripe sporocysts, inclosed in moist bread. Numerous vermicules were seen between the epithelial cells and in the capillaries and lymph spaces in the villi.

CLASSIFICATION.

Some zoologists have favored a classification of the gregarine-like blood parasites as a subdivision of the gregarines, while others have placed them in a subdivision of the *Hæmosporidia*, making blood parasitism the principal feature upon which to base the classification.

Just at present the question of their systematic position is in a somewhat confused state.

In the classification of Laveran[a] the *Hæmosporidia* are divided into three genera:

1. *Hæmamœba* (including the malarial type of organism).
2. *Piroplasma.*
3. *Hæmogregarina.*

Neveu-Lemaire[b] believes that these primary subdivisions should be into families instead of genera. He divides the *Hæmosporidia* into four families instead of three, viz:

Hæmogregarinidæ	*Lankesterella.*
	Karyolysus.
	Hæmogregarina.
Hæmamœbidæ	*Plasmodium.*
	Laverania.
	Hæmamœba.
Halterididæ	*Halteridium.*
	Polychromophilus.
Achromaticidæ	*Achromaticus.*
	Dactylosoma.

It has been suggested that *Piroplasma*, an important group which has been left out, be included as an additional family.

Lühe agrees with Neveu-Lemaire that the genus *Hæmogregarina* (Laveran) should be given the strength of a family.

The classification of Minchín[c] attempts to accomplish the same end in a somewhat different manner. It is a modification of one proposed by Labbé.

Hæmosporidia	*Hæmosporea*	*Lankesterella.*
		Karyolysus.
		Hæmogregarina.
	Acystosporea	*Plasmodium.*
		Laverania.
		Hæmoproteus.
		Halteridium.
		Piroplasma.

The description of the suborder *Hæmosporea* is as follows:

> Trophozoite, typically a vermiform hæmogregarine, endoglobular in early stages, free when full grown; apparently no alteration of hosts; schizogony and sporogony in the same host, which is always a cold-blooded vertebrate, fish, amphibian, or reptile.

The hæmogregarines of mammals could not be included in this suborder without considerable modification. The classification devised by Neveu-Lemaire and advocated by Lühe divides the *Hæmogregarinidæ* into three genera, as already mentioned.

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[a] Laveran, A.: Les hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 50, 1899, p. 124–133.

———: Essai de classification des hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 53, 1901, p. 798.

[b] Neveu-Lemaire: Les hématozoaires du paludisme. Thèse, Paris, 1901.

[c] Minchín: The sporozoa. In "A treatise on zoology," ed. by E. Ray Lankester, part 1, London, 1903.

In view of the restricted knowledge of many of the parasites of this general group, and especially in view of the fact that the complete life cycle for so few forms is definitely established, considerable difficulty naturally arises in attempting to definitely classify *Hepatozoon perniciosum,* the organism under discussion. As Lühe, who has recently published a revisional discussion of this group, has classified an apparently rather closely allied species, namely, *Hæmogregarina balfouri,* from *Jaculus jaculus* in the family *Hæmogregarinidæ,* it seems best to place this new species for rats at least provisionally in the same family.

In attempting to classily the organism in one of the new genera we are faced by two dangers: In the majority of the species of this group the complete life cycle has not yet been established. If, now, the organism under discussion is classified in one of these new genera the danger is present of giving a total misconception to the life cycle of the other forms. If, on the other hand, a new genus is proposed for this species the danger is present that when the life cycle of the other species of this group shall become known this new genus will be shown to be unnecessary. A choice must therefore be made between running the risk of confusing the biology of species with which the present parasite may have nothing whatever to do and of unnecessarily creating a new systematic name. When these considerations are weighed it would appear that it is much more difficult to correct a biological confusion than it is to suppress a synonym. On this account it is believed that the lesser of the two evils lies in proposing for the species under discussion a new generic name. Dr. Charles Waddell Stiles, chief of the division of zoology, Hygienic Laboratory, Public Health and Marine-Hospital Service has suggested the provisional formation of a new genus. The writer accepts this view, and accordingly proposes *Hepatozoon,* kindly suggested by Doctor Stiles as the new mono-typic genus, with *Hepatozoon perniciosum* as type species.

As only one species is at present established for this genus it is naturally difficult to separate the generic from the specific characters. As diagnosis for *Hepatozoon perniciosum* n. g., n. sp., the following may be given:

IN THE RAT.

When an infected mite is swallowed by a rat the sporocysts are acted upon by the digestive juices and the sporozoites are liberated. The sporozoites become actively motile striated vermicules measuring 16 by 4 micra. They penetrate the intestinal villi of the rat, enter the blood stream, and are carried to the liver. They penetrate the liver cells and undergo schizogony. The nuclei of the oval schizonts are arranged at the poles and an enveloping cyst is developed. Each nucleus becomes the basis of a merozoite, of which an average of 16 are formed, arranged at the poles. The ripe cyst measures 25 by 30 micra. Later the cyst wall becomes softened and is ruptured. The merozoites are set free as actively moving vermicules, indistinguishable from those developed from the sporozoites. Some of the merozoites enter fresh liver cells and repeat the schizogony. Other merozoites enter the blood streams as vermicules, are taken in by the large mononuclear lymphocytes, and develop a definite enveloping cyst wall. A few vermicules may escape the action of the leucocytes and appear in the blood stream.

In the Mite.

When the blood of an infected rat is swallowed by a mite the encysted trophozoites are set free in the stomach by solution of the cyst as free vermicules. Two similar vermicules become associated and conjugate. One, the macrogamete, grows larger and partly surrounds the other, the microgamete. The protoplasm becomes fused and later the nuclei conjugate and fuse to form a zygote. The zygote becomes a sluggishly motile ookinet, which penetrates the stomach wall of the mite and enters the body tissues and becomes encysted (oocyst). Here a remarkable enlargement of the karyosome takes place. The parasite increases enormously in size. The nucleus of the spherical sporont thus formed undergoes division into many daughter nuclei, which migrate to the surface of the sporont. The surface of the latter becomes mammillated. The projections, each of which contains a nucleus, increase in size and length; later they are broken off and each becomes a sporoblast. The nucleus of the sporoblast undergoes division, the resulting nuclei being arranged at the poles. The sporoblast increases in size and a cyst wall develops. Around each nucleus a sporozoite is formed. In the ripe sporocyst, which measures 25 by 30 micra, the sporozoites, 16 in number (average), are arranged at the poles. The large cyst (oocyst) contains from 50 to 100 of such sporocysts. When the mite is swallowed by a rat the cycle is repeated.

It is recognized that the hæmogregarines *Leucocytozoon canis* and *Leucocytozoon funambuli*, which have already been described, in India are more or less similar to *Hepatozoon perniciosum;* but as the genus *Leucocytozoon* has already been taken by a blood parasite, *L. ziemanni,* of an entirely different character, this generic name can not be properly retained.

The relation or possible identity of *Leucocytozoon ratti,* described by Adie, with *H. perniciosum* can only be cleared up when the life cycle of the former is known. Only the trophozoite has been observed, which, according to the description, is smaller than that of *H. perniciosum*, and is without a cyto-cyst. It may be mentioned that there exists a marked similarity in form of the trophozoites of different species of hæmogregarines in both warm- and cold-blooded animals.

Specimens of the type species of the new genus have been deposited in the museum of the division of zoology, hygienic laboratory, United States Public Health and Marine-Hospital Service.

CONCLUSIONS.

Hepatozoon perniciosum is the only hæmogregarine occurring in mammals which has been observed outside of India and northern Africa, both tropical regions.

No other hæmogregarine has been described which exhibits distinct pathogenic properties or causes the death of the animal host.

Other hæmogregarines have been found only in wild animals, in which they appear to exert no harmful effect. It is well known that domesticated animals may succumb to infection by protozoa or bacteria, practically harmless to closely related wild animals. Such may be the case as regards infection of white rats by *H. perniciosum*.

The results thus far obtained in the experimental infection of wild rats (*Mus rattus*) with this parasite are not conclusive as regards pathogenicity. That the wild rat is susceptible to infection is clearly shown. As is the case with white rats, it is probable that the most pathogenic effect is exerted upon young animals. Whether or no this parasite can be turned to account in the destruction of wild rats is a matter for further investigation. The role assumed by wild rats as agents in the transmission of plague makes it a matter of great importance to exterminate them. To do this by spreading disease among them is generally regarded as the simplest and most desirable method.

The Gamasid mite (*Lelaps echidninus*), which is the intermediate host, has frequently been found both upon white and wild rats and in widely separated localities. A study of the habits of rats taken in conjunction with the experimental infections already described, proves beyond question the mode of entry of *Hepatozoon perniciosum* into the body of the rat. That this is the sole manner in which infection takes place is not proved, but is extremely probable.

The puncture produced by the pointed mouth parts of the mite before sucking the blood of the host apparently causes some irritation. Rats infested with mites may frequently be seen to bite at the skin on various parts of the body and frequently they seem to catch and devour the offending mite. The behavior of dogs when disturbed by fleas is more familiar.

If the mite in question has previously sucked blood from a diseased animal and sufficient time has elapsed for the development of sporocysts, the latter, when dissolved in the intestinal juice of the rat, set free the sporozoites. These as vermicules penetrate the intestinal wall, enter the veins and lymphatics, and reach the liver. Here they penetrate the liver cells and undergo multiplication. Some escape into the general circulation. The merozoites when fully developed are set free in the liver capillaries as vermicules. The majority are speedily taken up by the phagocytic large lymphocytes and become encysted: a few may remain as free vermicules.

The habit of mites to leave the rat during the day and return at night affords ample opportunity for a change of hosts if several animals are in the same cage.

As shown by experiment, in rats fed upon infected mites mixed with bread, free vermicules appear in the blood twenty-four hours later and encysted forms after eight or ten days, whereas rats upon which live infected mites are placed show infection only after seventeen to twenty-eight days. The explanation of this difference doubtless depends upon how soon after being placed on the rat an infected mite is devoured.

As already mentioned in the description of the parasite in the body of the mite, there is no indication, either in preparations of fresh mites or in prepared sections, that the sporozoites become free from the sporocysts in the body of the mite or that they are transmitted by the mouth parts. The sporocysts show considerable tenacity and resistance to rupture. Although sometimes encountered in the salivary glands, the sporocysts are common in any part of the body of the mite.

Infection through the alimentary canal from an intermediary host has not previously been described for a protozoan blood parasite.

The fact that all of those animals, viz. frogs, snakes, lizards, turtles, etc., in which hæmogregarines are so common, are insectivorous suggests a possible mode of infection the same as in white rats. *Leucocytozoon canis*, the sexual cycle of which Christophers has described in the dog tick, may possibly be transmitted in the same manner as *H. perniciosum*.

The limited experiments seem to indicate that infection with the latter by inoculation from one animal to another is impossible.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus: an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car santitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous zoological papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*).—By W. W. Miller.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

MAILING LIST.

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The editions of the publications marked (*), available for distribution by the Surgeon-General of the Public Health and Marine-Hospital Service, have been exhausted. Copies may, however, be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., who sells publications at cost and to whom requests for publications thus marked should be made.

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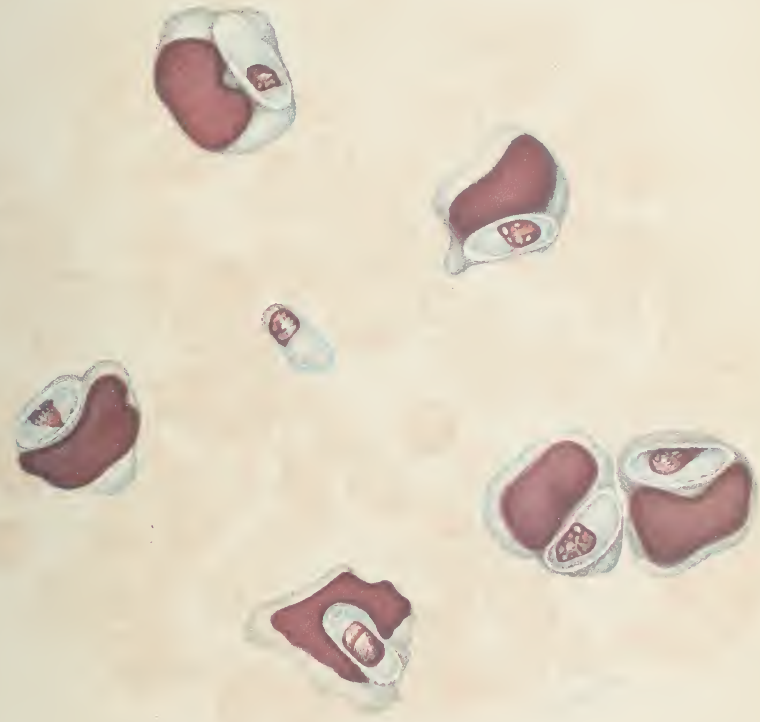
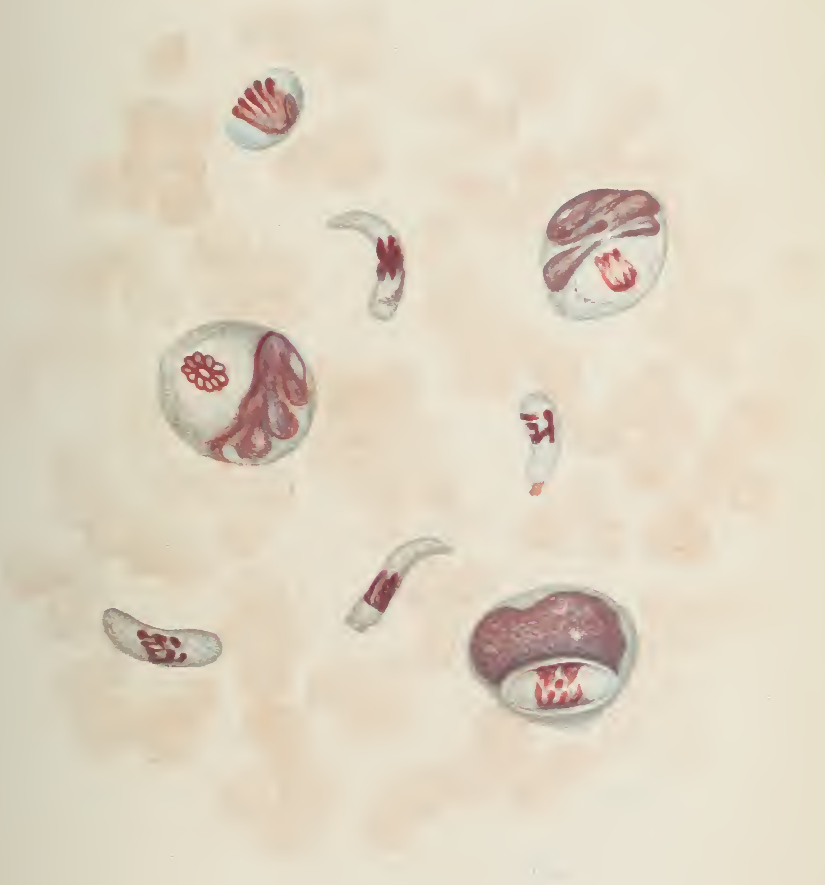


A Hoen & Co.Baltimore.

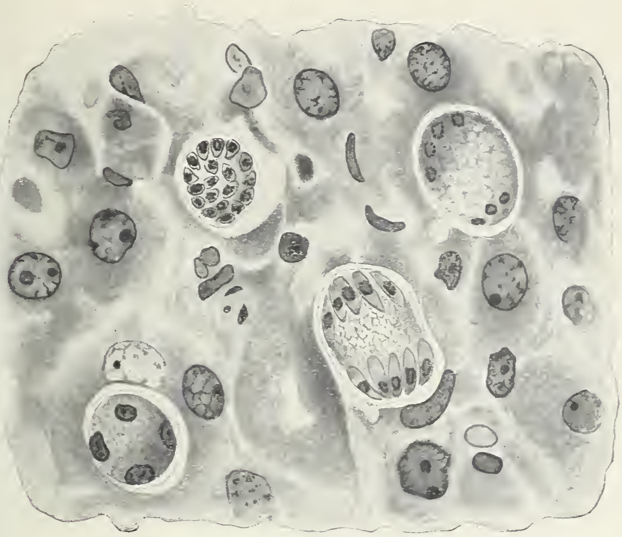
Blood smear showing *Hepatozoon perniciosum* encysted in large lymphocytes; also, one free encysted form. Drawn with the aid of the camera lucida from a single field of the microscope. Giemsa stain.



A Hoen & Co. Baltimore

Blood smear showing unusual forms of *Hepatozoon perniciosum.* *a.* free vermicules; *b.* motionless, free parasites; *c.* large oval parasite without cyst; *d.* large parasite in transitional leucocyte, unencysted; *e.* ordinary size parasite in transitional leucocyte, without cyst; *f.* large parasite encysted in large mononuclear lymphocyte.

Drawn with camera lucida. Giemsa stain.



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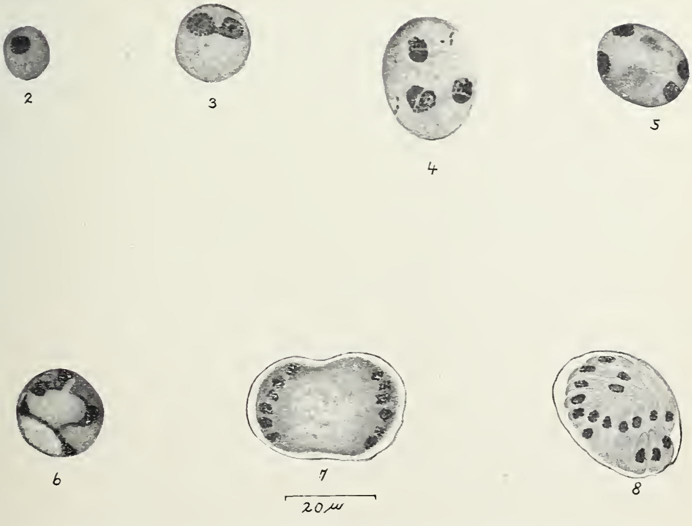
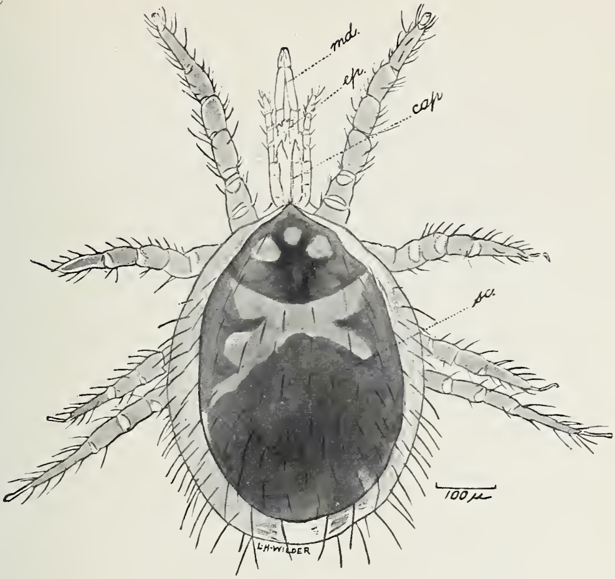


Fig. 1.—Section of liver of infected rat, showing schizonts in various stages and the formation of merozoites. Figs. 2–8.—Detail drawings of schizonts in different stages (from a section of the liver). Drawn with camera lucida. Stain, iron hematoxylin.



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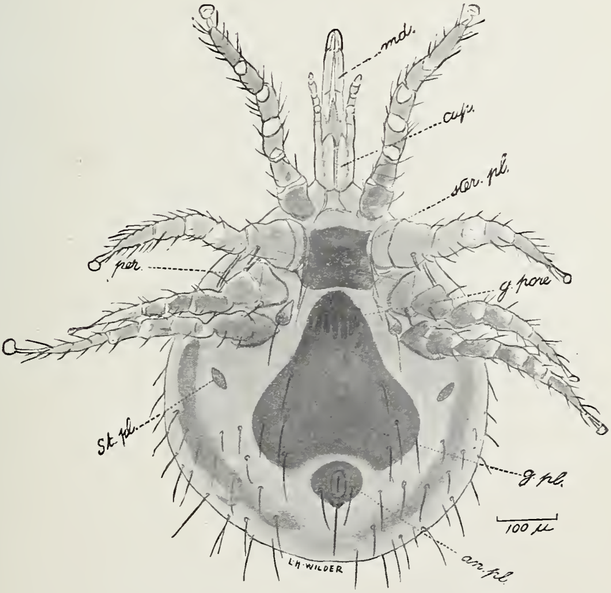
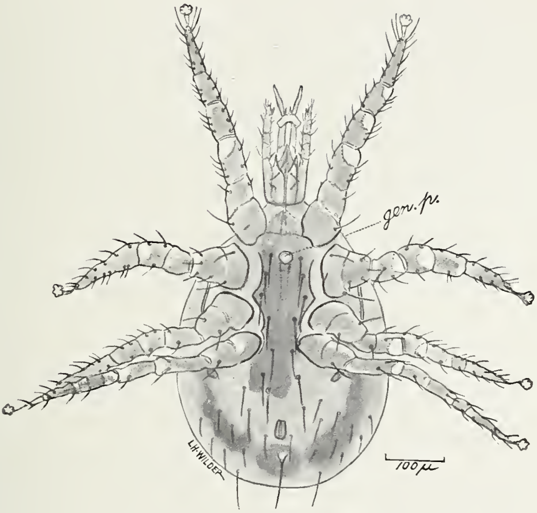


2

Fig. 1.—Dorsal view of a female mite. Fig. 2.—Ventral view of a female mite. *md.*, mandibles; *ep.*, epistome; *cap.*, capitellum; *sc.*, scutum; *per.*, peritreme; *st. pl.*, stigmal plate; *g. pl.*, genital plate; *an. pl.*, anal plate; *g.*, genital pore; *ster. pl.*, sternal plate. Drawn with camera lucida.



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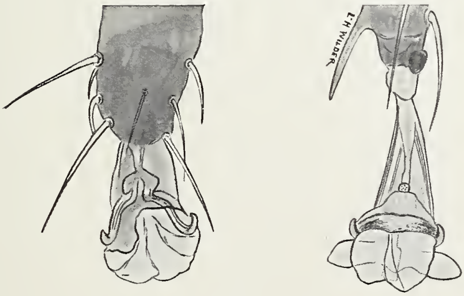
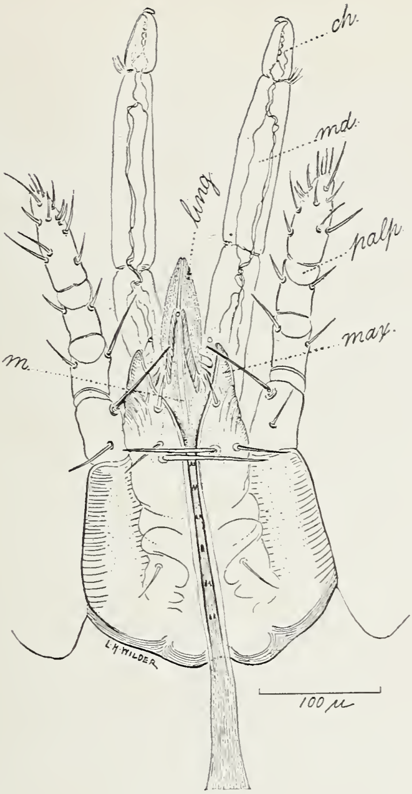


2

Fig. 1.—Ventral view of male mite. *gen. p.*, genital pore. Drawn with camera lucida. Fig. 2.—Sucking disks and hooklets upon terminal phalanx of first pair of legs. Greatly enlarged.

PLATE VI.



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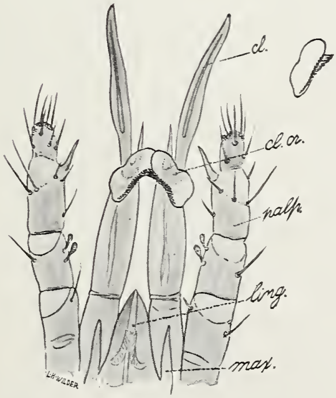
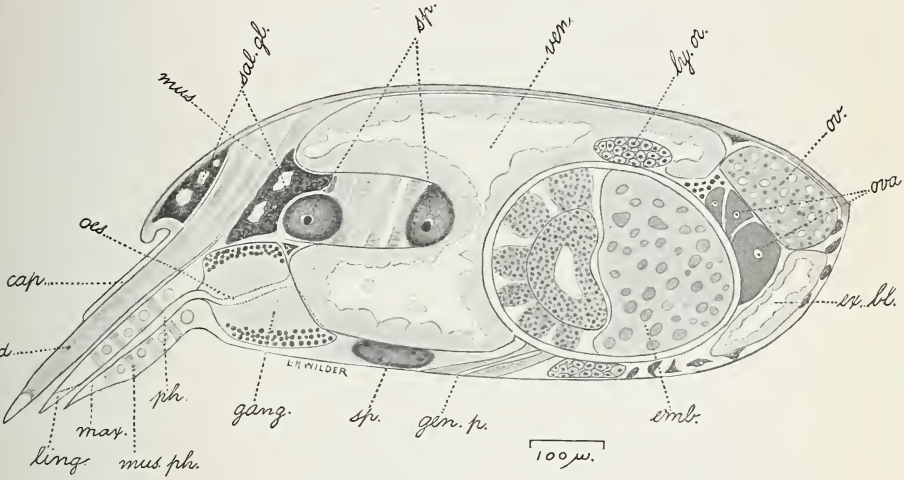


2

Fig. 1.—Mouth parts of female mite. Fig. 2.—Mouth parts of male mite. *ch.*, cheliceræ; *md.*, mandible; *palp*, palp; *max.*, maxilla; *m.*, mouth opening; *cap.*, capitellum; *ling.*, lingula; *cl. or.*, clasping organ. Greatly enlarged. Drawn with camera lucida.



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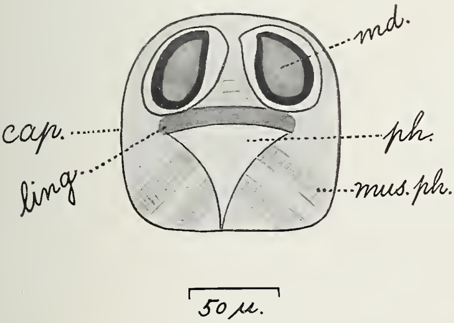

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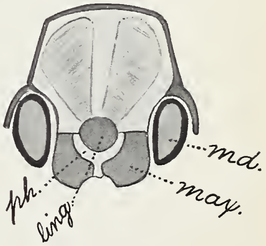
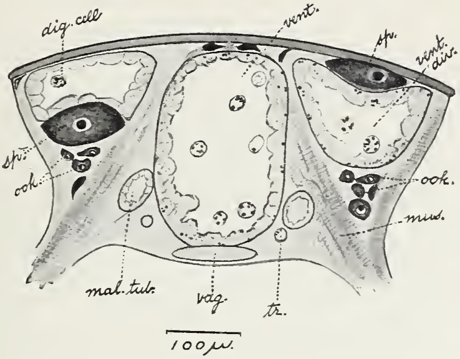


3

Fig. 1.—Dorsi-ventral section in mid line of female mite. Semidiagrammatic. Fig. 2.—Transverse section of posterior part of capitellum. Fig. 3.—Transverse section of anterior part of capitellum (enlarged). *ven.*, ventriculus; *cap.*, capitellum; *ov.*, ovary; *ova*, ova; *emb.*, embryo; *gang.*, ganglion or brain; *gen. p.*, genital pore; *œs.*, œsophagus; *sal. gl.*, salivary gland; *ly. or.*, lyrate organ; *mus.*, muscle fibers (attached to mandible, and under surface of scutum); *md.*, mandible; *max.*, maxilla; *ex. bl.*, excretory bladder; *ling.*, lingula; *ph.*, pharynx; *mus. ph.*, pharyngeal muscles; *sp.*, sporont (parasites).



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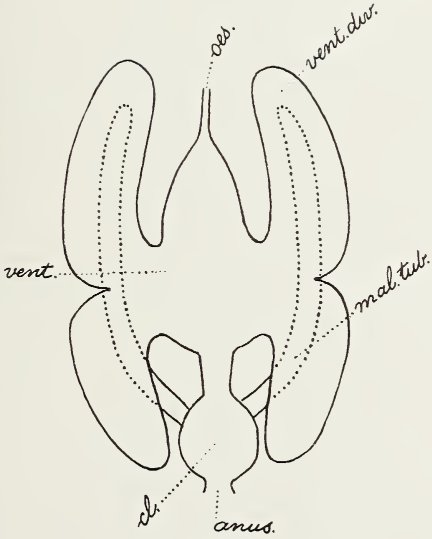
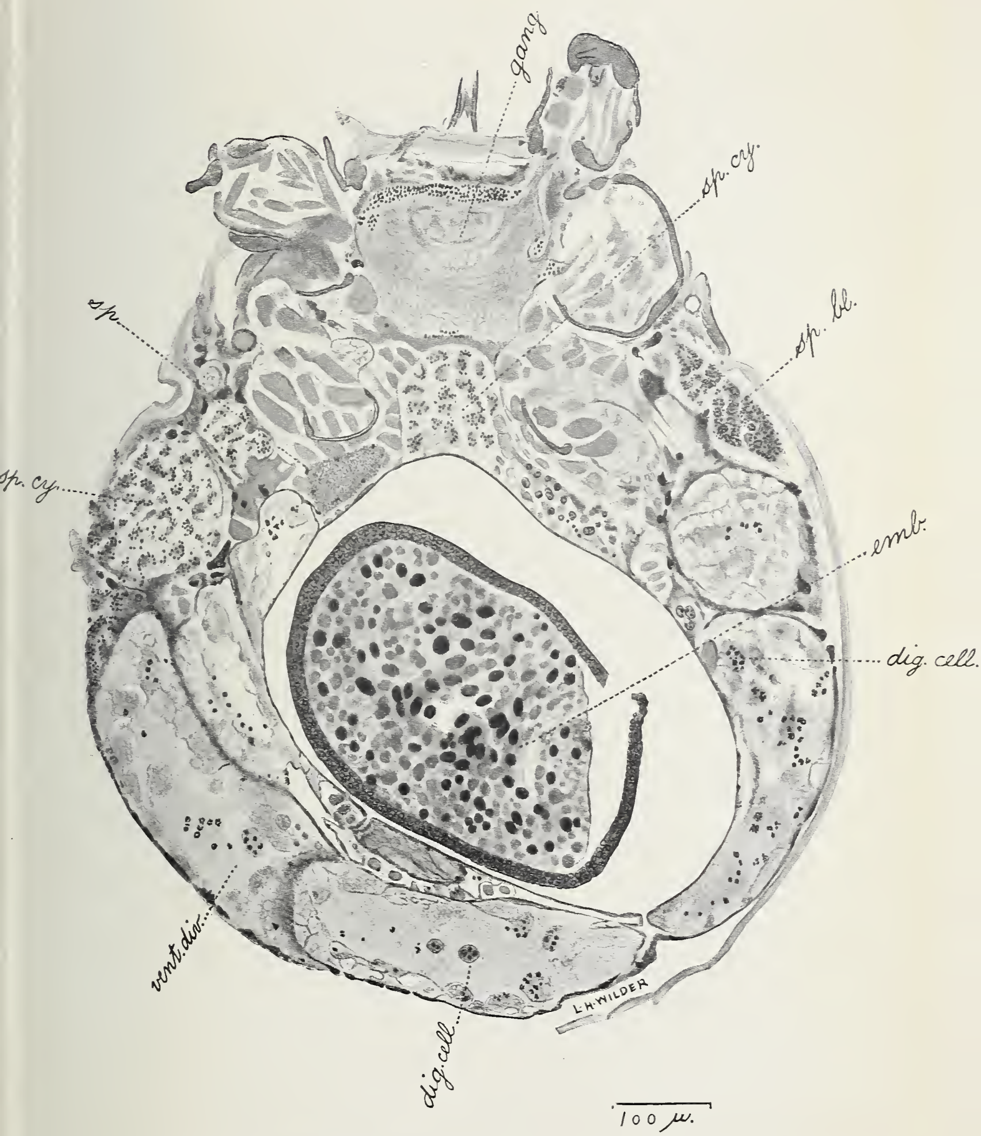
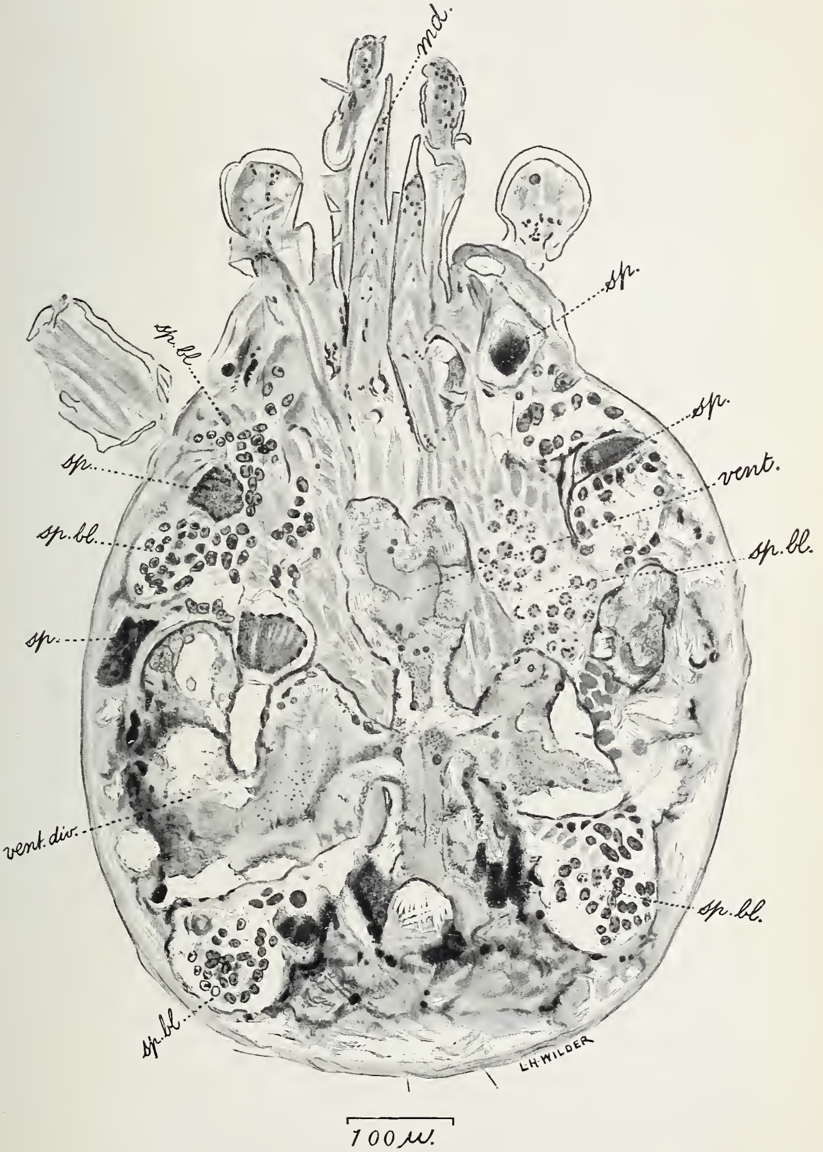


2

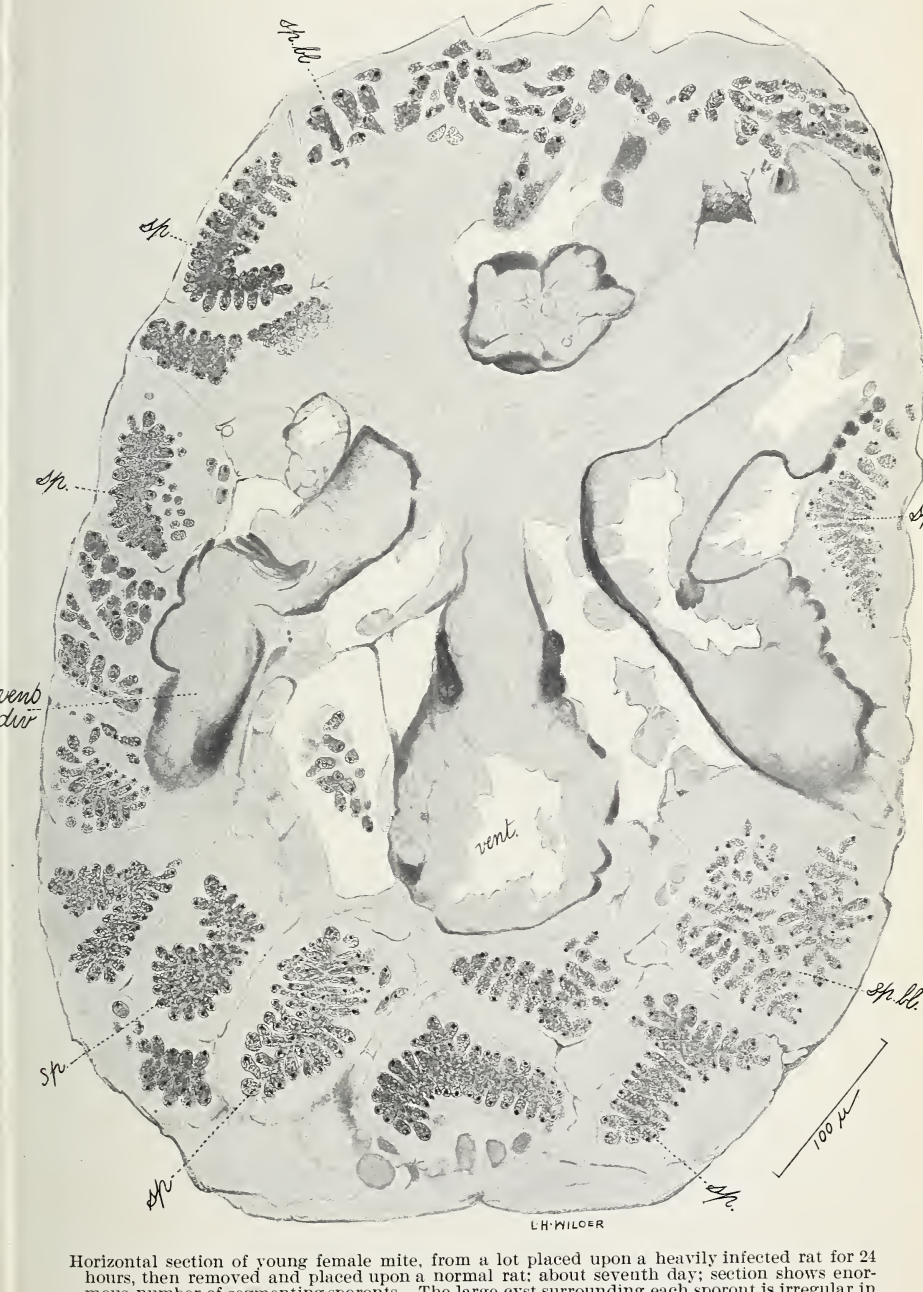
Fig. 1.—Transverse section of female mite, just behind fourth pair of legs. Semidiagrammatic. *dig. cell*, digestive cell; *vag.*, vagina; *tr.*, trachea; *mus.*, muscle attached to legs; *sp.*, sporont: *ook.*, ookinet. Fig. 2.—Diagram of digestive tract and excretory organs. *vent.*, ventriculus; *vent. div.*, ventricular diverticulum; *mal. tub.*, malphigian tubule; *cl.*, excretory bladder; *œs.*, œsophagus.



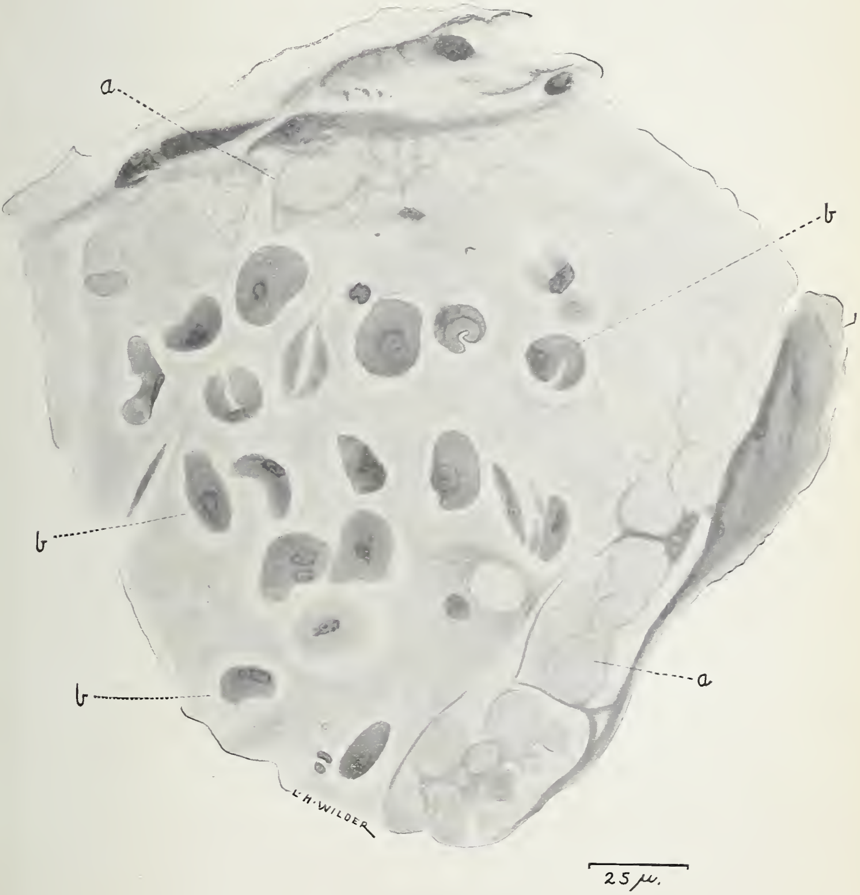
Horizontal section of female mite, removed from an infected rat. *sp. cy.*, large cyst containing many sporocysts with sporozoites—the cyst walls do not show distinctly—the nuclei of the sporozoites are strongly stained: *sp.*, sporont showing peripheral nuclei; *sp. bl.*, large cyst containing sporoblasts; *gang.*, ganglion or brain; *vent. div.*, ventricular diverticulum; *dig. cell*, digestive or liver cell; *emb.*, embryo. Drawn with camera lucida. Stained with iron hematoxylin.



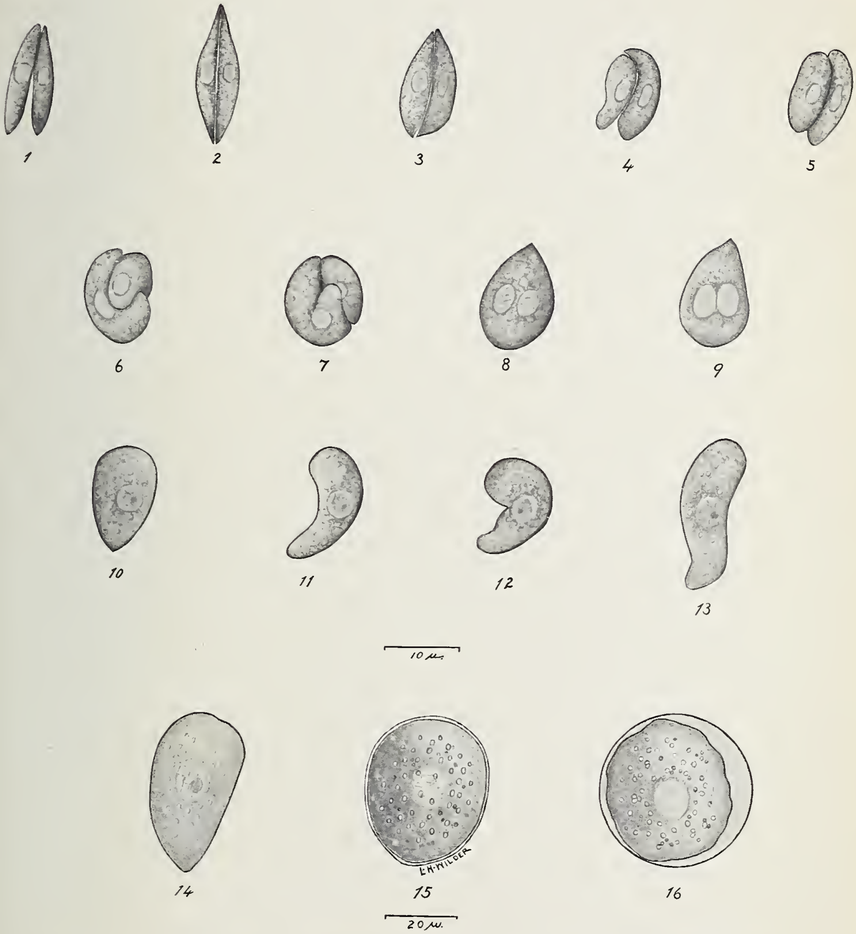
Horizontal section of young female mite from an infected rat, showing parasites. *sp. bl.*, sporoblasts (not yet divided into sporozoites) in large cysts, with indistinct outline; *sp.*, sporont. Lettering as in Pls. V to IX. Drawn with camera lucida. Stained with iron hematoxylin.



Horizontal section of young female mite, from a lot placed upon a heavily infected rat for 24 hours, then removed and placed upon a normal rat; about seventh day; section shows enormous number of segmenting sporonts. The large cyst surrounding each sporont is irregular in outline. Some of the sporonts are shown completely divided up into sporoblasts. Drawn with camera lucida. Stained with iron hematoxylin.

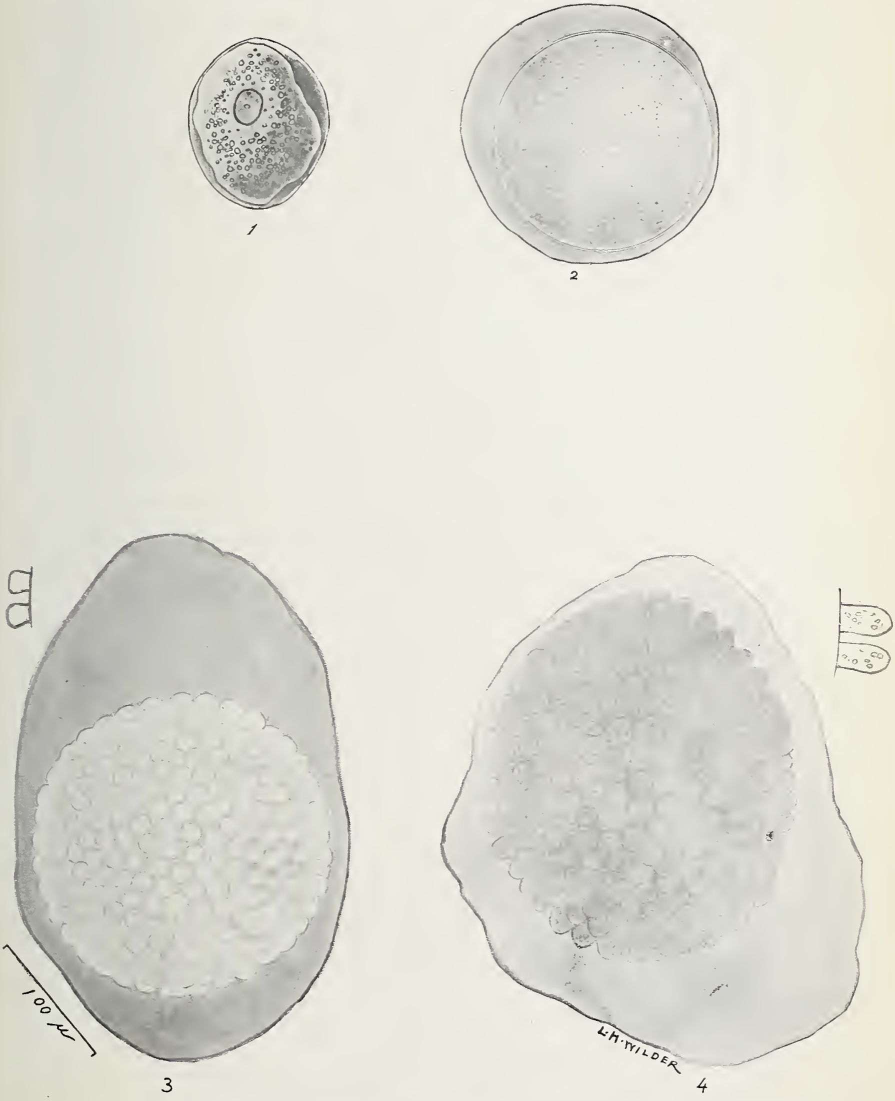


From a horizontal section of a mite 48 hours after feeding upon an infected rat. Figure represents the anterior portion of the left lateral diverticulum of the ventriculus in section. Stained with iron hematoxylin. *a*, Epithelial and muscular layer of ventriculus; *b*, zygotes and ookinets of various forms. Drawn with camera lucida.



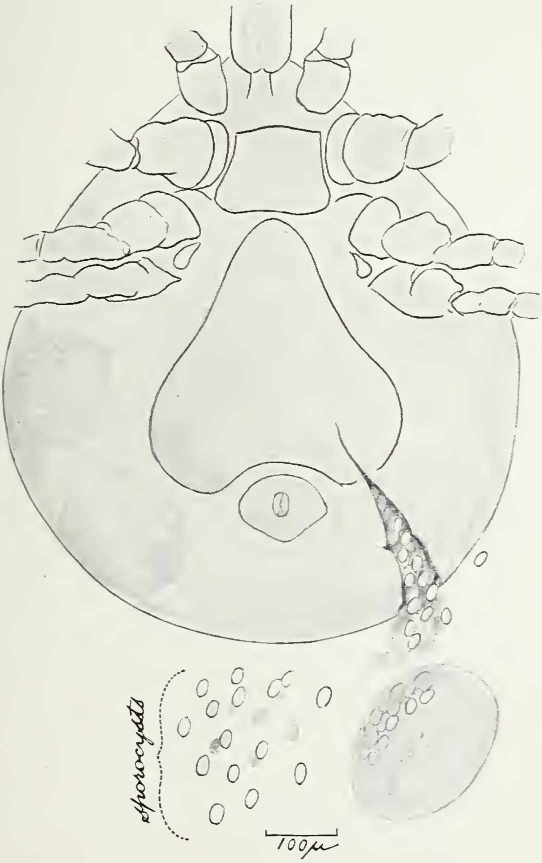
SEXUAL PHASE OF HEPATOZOON PERNICIOSUM IN THE STOMACH AND BODY TISSUES OF A FEMALE MITE.

Fig. 1.—Association of vermicules (gametocytes). Figs. 2-5.—Conjugation of gametes. Figs. 6-9.—Copulation of gametes to form a zygote. Figs. 10-14.—Formation and development of ookinet. Figs. 15-16.—Formation of an enveloping cyst and beginning of sporont stage. Figs. 1-12.—In the ventriculus. Figs. 13-16.—In the body tissues. Drawn from fresh preparations.

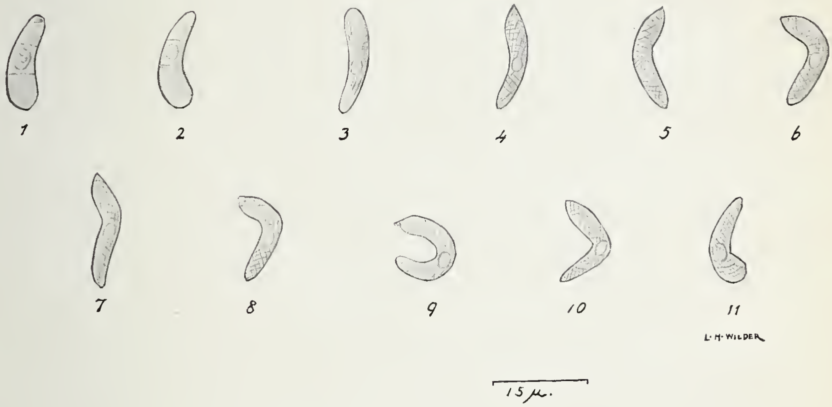


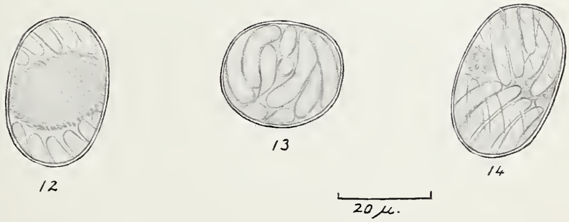
SHOWING SPORONTS IN VARIOUS STAGES, IN FRESH PREPARATIONS, MADE BY TEASING APART LIVE MITES FROM 3 TO 6 DAYS AFTER INFECTION, IN 0.3 PER CENT SALT SOLUTION.

Figs. 1-2.—Unsegmented sporonts with cyst. Figs. 3-4.—Sporonts showing budding from surface to form sporoblasts; with cyst. Drawn with camera lucida.

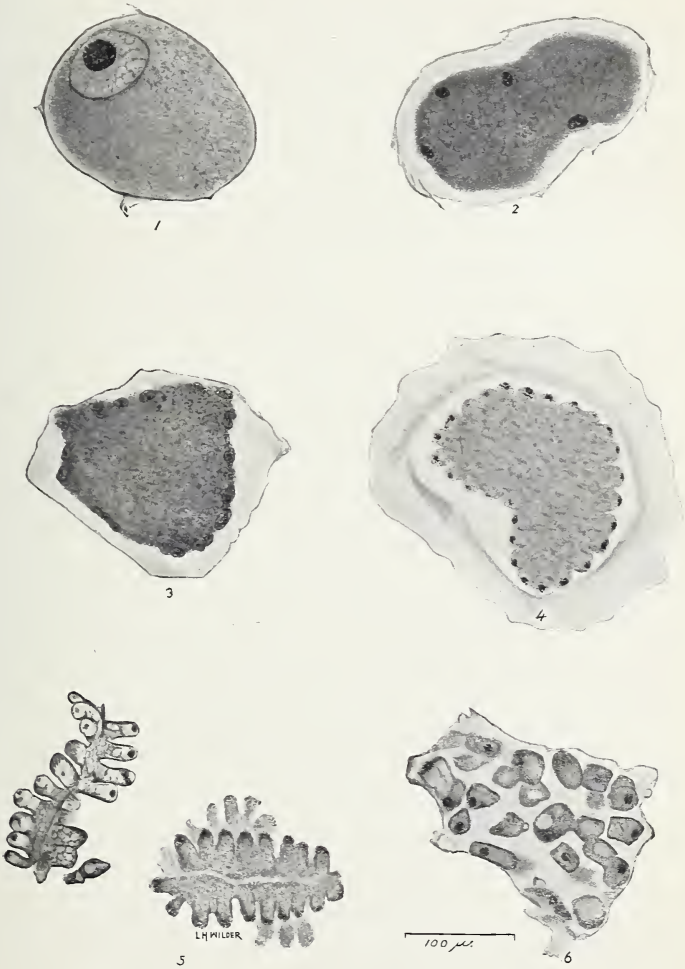


A MITE CONTAINING 3 LARGE CYSTS, WHICH HAS BEEN GENTLY CRUSHED IN SALT SOLUTION BETWEEN SLIDE AND COVER SLIP. SHOWING EXTRUSION OF A LARGE CYST CONTAINING MANY SPOROCYSTS; AND MANY FREE SPOROCYSTS. FRESH PREPARATION. SEMIDIAGRAMMATIC.





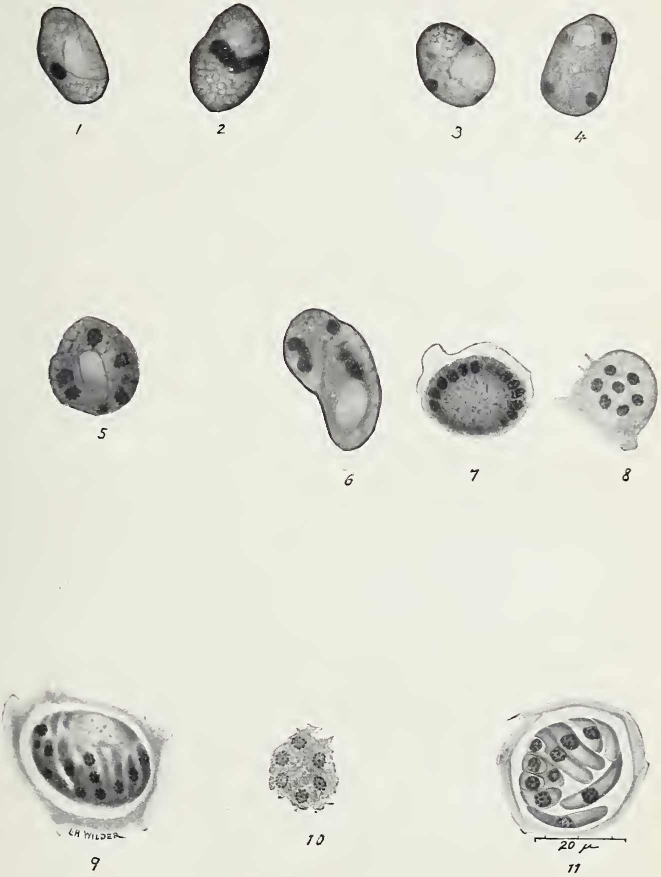
Figs. 1-11.—Free vermicules. Drawn from a fresh preparation, prepared by mixing ripe sporocysts from a crushed mite with duodenal juice obtained from a freshly killed healthy rat. Figs. 1-2.—Sporozoites unchanged. Figs. 3-11.—Sporozoites which have become active vermicules; showing striation (exaggerated) and various shapes assumed. Fig. 12.—Sporocyst from freshly crushed mite; showing central "rest" body and young sporozoites at the extremities. Figs. 13-14.—Lateral and end views of fresh sporocysts, showing completely developed sporozoites and small "rest" body.



PARASITES DRAWN FROM SECTIONS OF MITES. STAINED WITH IRON HEMATOXYLIN.

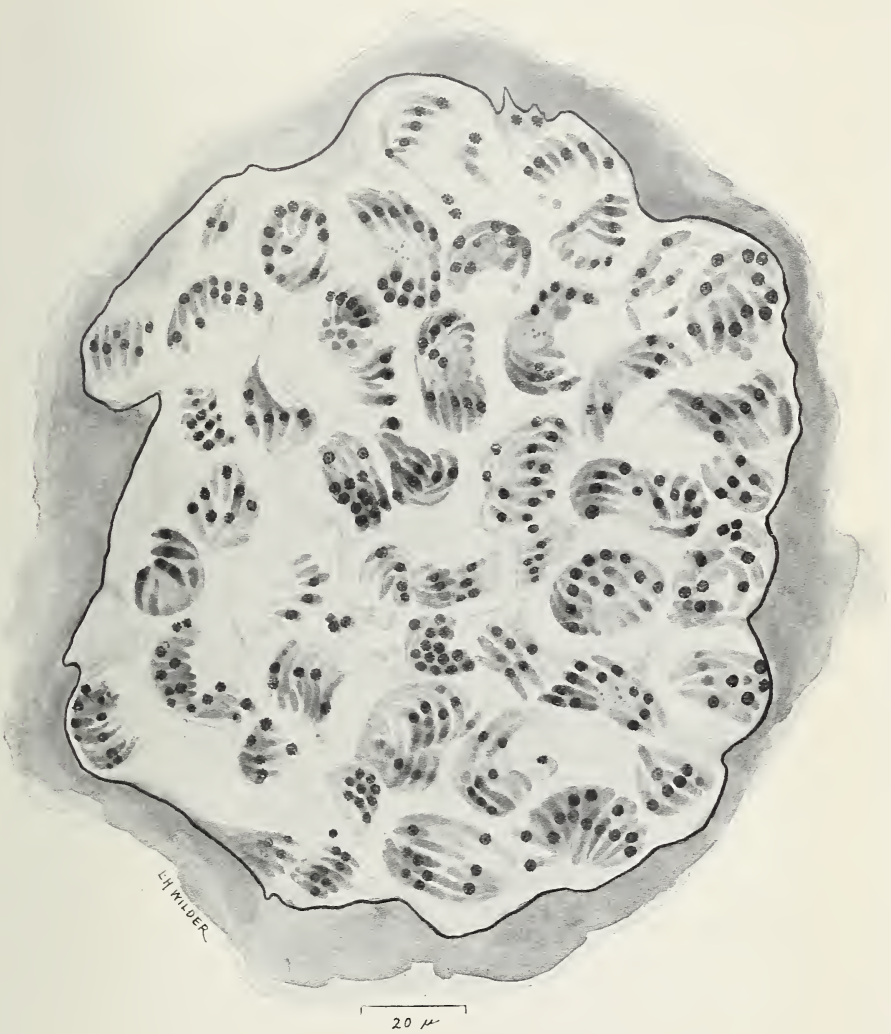
Fig. 1.—Large sporont. Figs. 2–3.—Sporonts with daughter nuclei. Fig. 4.—Early bud formation. Fig. 5.—Segmentation into sporoblasts almost complete. Fig. 6.—Complete segmentation into sporoblasts. Stages shown in figs. 4, 5, and 6 are numerous in Pl. XI. Drawn with camera lucida.

PLATE XVIII.

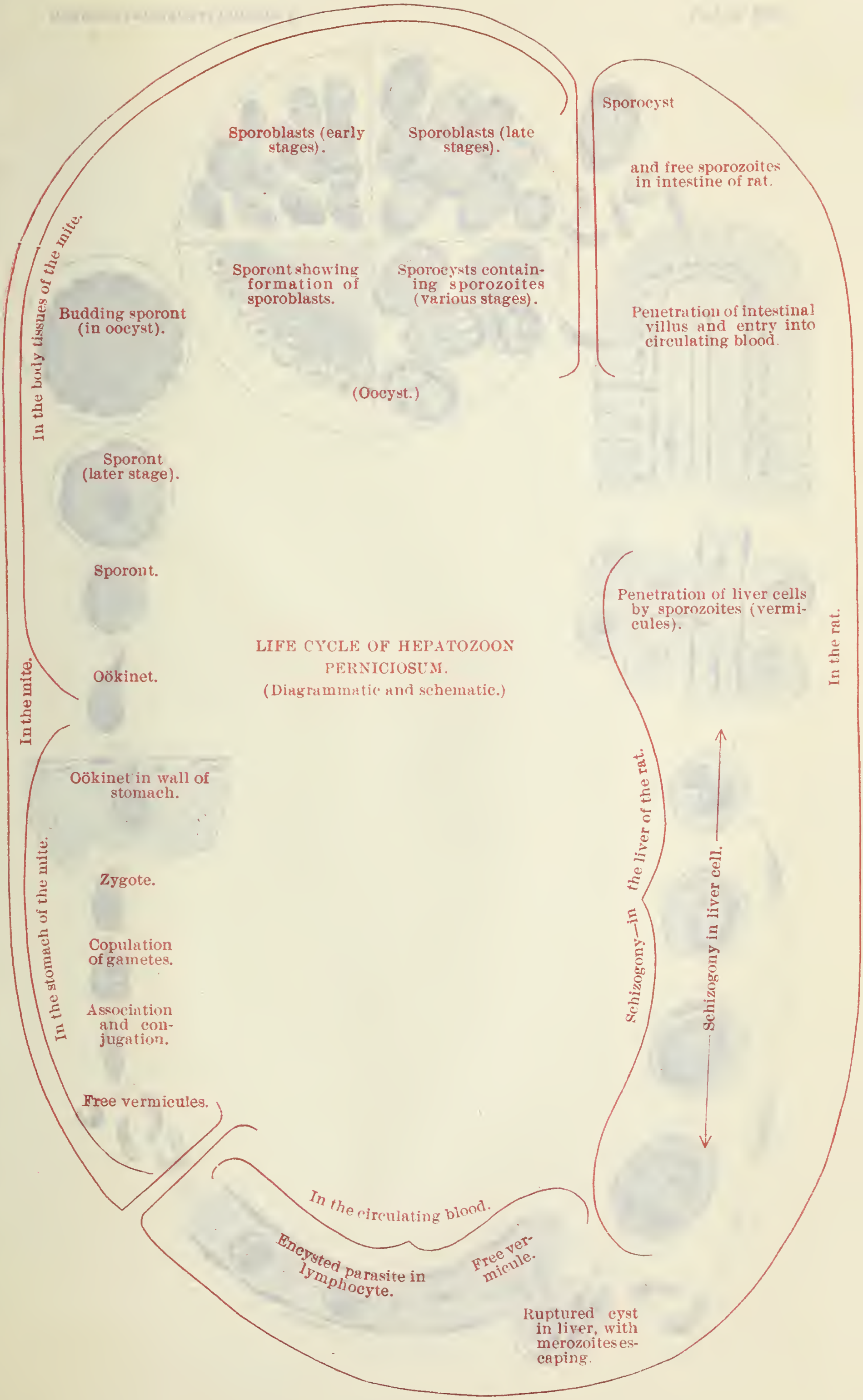


SPOROBLASTS SHOWING VARIOUS STAGES OF NUCLEAR DIVISION AND SEGMENTATION INTO SPOROZOITES; FROM SECTIONS OF INFECTED MITES, 7 TO 10 DAYS AFTER BEING PLACED UPON INFECTED RATS. STAINED WITH IRON HEMATOXYLIN.

Figs. 8 and 10 show cross sections of sporoblasts near one extremity. Drawn with camera lucida.

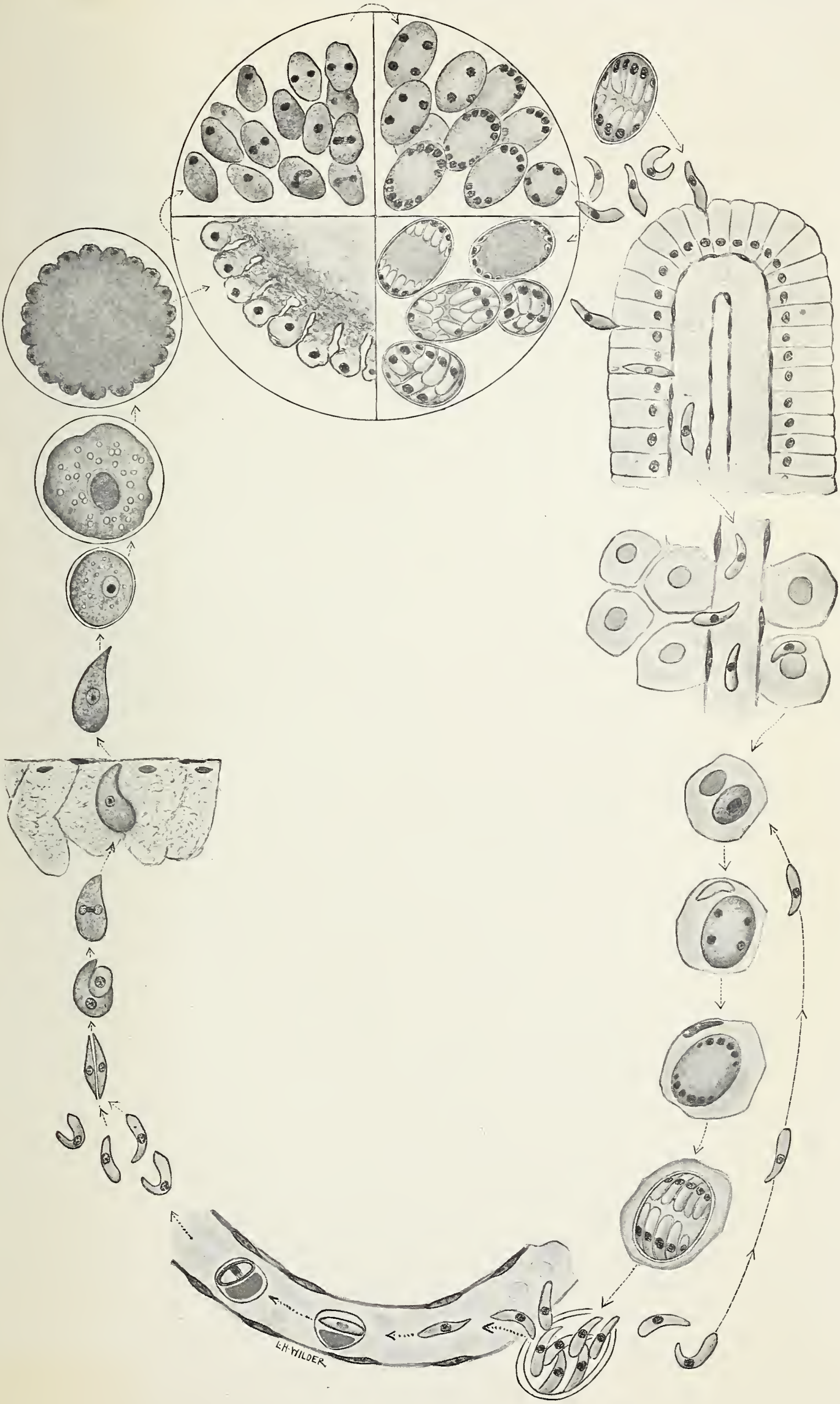


CROSS SECTION OF LARGE OOCYST (FROM SECTION OF A MITE) CONTAINING MANY RIPE SPOROCYSTS WITH SPOROZOITES. STAINED WITH IRON HEMATOXYLIN. DRAWN WITH CAMERA LUCIDA.


LIFE CYCLE OF HEPATOZOON PERNICIOSUM.
(Diagrammatic and schematic.)

Sporoblasts (early stages).
Sporoblasts (late stages).
Sporocyst
and free sporozoites in intestine of rat.
In the body tissues of the mite.
Budding sporont (in oocyst).
Sporont showing formation of sporoblasts.
Sporocysts containing sporozoites (various stages).
Penetration of intestinal villus and entry into circulating blood.
(Oocyst.)
Sporont (later stage).
Sporont.
Penetration of liver cells by sporozoites (vermicules).
In the rat.
Oökinet.
In the mite.
Oökinet in wall of stomach.
In the stomach of the mite.
Schizogony—in the liver of the rat.
Schizogony in liver cell.
Zygote.
Copulation of gametes.
Association and conjugation.
Free vermicules.
In the circulating blood.
Encysted parasite in lymphocyte.
Free vermicule.
Ruptured cyst in liver, with merozoites escaping.

PLATE XX.



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